University of Alberta

Mapping the introgression of the *Brassica carinata* C and B genomes into *Brassica napus*

by

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A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy
in
Molecular Biology and Genetics

Department of Biological Sciences

Edmonton, Alberta
Spring 2008

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Your file Votre référence
ISBN: 978-0-494-45609-5
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ISBN: 978-0-494-45609-5

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Abstract

The *Brassica* are a genus containing valuable vegetable, fodder and oilseed crops that are cultivated on a large scale on all five continents. The B genome containing *Brassica* (*B. nigra, B. juncea* and *B. carinata*) carry a number of valuable genetic traits including tolerance to abiotic stresses, and resistance to a number of important pathogens of *B. napus*- which is the species most commonly used to produce canola quality oil. Several attempts have been made to introgress B genome traits from these species into *B. napus*, but they have not resulted in the stable integration of B genome resistance into *B. napus*. This thesis describes why B genome introgressions have been unsuccessful. Two interspecific lineages derived from crosses between *B. napus* (AACC) and *B. carinata* (BBCC) were developed, and microsatellite markers were used to monitor the inheritance of the C and B genomes through four generations of introgression breeding. The marker data revealed that B genome chromosomes do not undergo recombination with the A or C genomes of *B. napus*. Instead, B genome chromosomes were maintained as whole non-recombining chromosomes with the occasional loss of terminal chromosomal regions through successive meioses. The exception was a small terminal region of B5/J15 that was introgressed via translocation into *B. napus* (A /C) during a meiotic event of an F1 hybrid (ABCC). Recombination between the C genomes of *B. carinata* and *B. napus* did occur, and were two-fold higher than values observed in intraspecific crosses. This research suggests that transferring B genome traits to the *Brassica* A or C genomes would be impractical unless the desired trait was a single gene/single locus trait terminally located on a B genome chromosome. Conversely,

traits located on the C genome of *B. carinata* could be reliably introgressed into the *B. napus* genome.

Acknowledgements

I would like to thank my supervisor, Dr. Allen Good, for his guidance and for the opportunity to conduct scientific research towards a Ph.D. at the University of Alberta. I would also like to thank Dr. Derek Lydiate (AAFC Saskatoon) for several informative discussions regarding this project, access to the AAFC resources, and many nights of board games in Saskatoon.

The following people were also of great help to me:

Dr. Roger Rimmer (AAFC Saskatoon) supplied the *L. maculans* isolates and expertise for blackleg screening.

Erin Higgins (AAFC Saskatoon) provided much appreciated technical mentoring during the initial stages of molecular data generation.

Rebecka Carroll (U of A) helped me with 600+ grueling genomic DNA extractions.

Yvone Pearson (U of A) who aided with the seed harvest of many hundreds of plants.

Dr. Reinhold Mayerhofer (U of A) for general amusement and technical discussions.

I would like to thank the U of A Biological Sciences Web Administrator-guy for installing Genographer and other hard-to-install programs I required, and then marrying me. Your support has been, and will always be, cherished.

I am also generally grateful for the generosity of my family, for all of the energetic friends I have found in Edmonton, and for the focus/ distraction being able to play Ultimate has provided me.

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List of Abbreviations

AAFC	Agriculture and Agri-Food Canada resources
Avr	avirulence gene
BA	benzol aminopurine
BC1	first generation backcross
BC2	second generation backcross
BC3	third generation backcross
bp	base pairs
cM	centiMorgan
CMS	cytoplasmic male sterility
EST	expressed sequence tag
F1	hybrid generation (first generation)
F2	second selfed generation
GA3	gibberellic acid
HR	hypersensitive response
IBA	indole butyric acid
LG	linkage group
LOD	logarithm of the odds (to the base 10)
Mi	root-rot resistance gene
MS	Murashige and Skoog plant tissue culture media
m.u.	map units
Myr	million years
NAA	naphthalene acetic acid
PCR	polymerase chain reaction
PG	pathogenicity group
*p*RF	*pseudo*-recombination frequency
RF	recombination frequency
RFLP	restriction fragment length polymorphism
Rfo	nuclear restorer locus
SG	screening gel
SSR	short sequence repeat- microsatellite marker

1. General Introduction

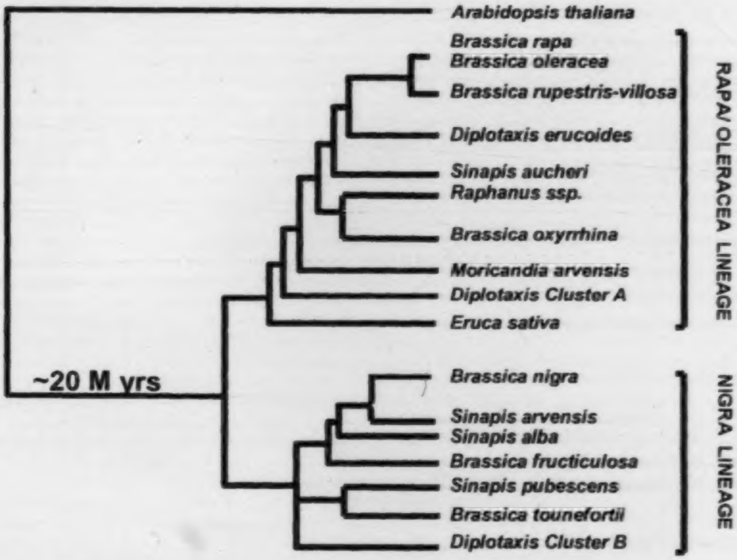
1.1. The *Brassicaceae* family and the genus *Brassica*

The *Brassicaceae* (formally Cruciferae) include approximately 338 genera and almost 3700 species of plants, organized into 25 tribes (Johnston *et al.*, 2005; Beilstein, *et al.*, 2006). The *Brassica* genus alone comprises approximately 100 species, of which many are important vegetable, oilseed and food sources (Al-Shehbaz, 1973). The genus is native to Western Europe, the Mediterranean and temperate regions of Asia. In addition to the cultivated species, which are grown worldwide, many of the wild species grow as weeds, especially in North America, South America, and Australia. Almost all parts of the *Brassica* plants have been developed for human food, including the root (swede or yellow turnip; *B. napus*), stems (kohlrabi; *B. oleracea*), leaves (cabbage, brussels sprouts; *B. oleracea*), flowers (cauliflower, broccoli; *B. oleracea*), and seeds (mustard seed, oilseed rape and canola oil; *Sinapis alba*, *B. rapa*, *B. napus*). Some forms are also grown for ornamental purposes (kale, *B. oleracea*).

Morphologically, these dicotyledonous plants are characterized by a main flowering raceme, followed by other branching racemes, early leaves which tend to be lobe shaped and attached to the stem in an alternate fashion by a stalk, and later leaves which are longer, narrow, tapering and have a saggitate base wrapping around the stem. *Brassica* species produce characteristic four lobed perfect flowers ranging in color from purple, to white or yellow, with four sepals and six anther bearing stamens. Fertilized flowers give rise to long, slender seed bearing siliques (pods). Crop varieties of *Brassica* are self-fertile; however self-incompatibility is common in natural populations of the group (Ekuere *et al.*, 2004).

In Canada, *Brassica napus*, *Brassica rapa,* and *Brassica juncea* are used as sources of edible canola quality oil. In addition, a variety of *Brassica oleracea* sub-species are sources of a number of vegetable crops. On a smaller scale, *Brassica nigra* and the related species *Sinapis alba* (Figure 1.1) are used as a source of condiment mustard. Some races of *Brassica* have also become naturalized weeds such as wild mustard and wild rape (*B. napus, B. nigra* and *B. rapa*).

The various members of the *Brassica* genus are remarkably closely related (U, 1935; Figure 1-2). The genera were initially divided into two major lineages, the *B. oleracea/B. rapa* group and the *B. nigra* group (Warwick *et al.*, 1992). More recently, 16 of the 25 tribes were further subdivided into 3 lineages (referred to as Lineages I-III; Beilstein *et al.*, 2006). The classifications in Figure 1-1 were made using chloroplast DNA restriction site variation (RFLP) (Warwick *et al.*, 1992) and although incomplete, it provides a useful reference for comparative studies within the family. The presence of species from the *Sinapis, Diplotaxis* and *Brassica* genera in both lineages attest to difficulties in making the original classifications based on morphology.



Arabidopsis thaliana
Brassica rapa
Brassica oleracea
Brassica rupestris-villosa
Diplotaxis erucoides
Sinapis aucheri
Raphanus ssp.
Brassica oxyrrhina
Moricandia arvensis
Diplotaxis Cluster A
Eruca sativa
Brassica nigra
Sinapis arvensis
Sinapis alba
Brassica fructiculosa
Sinapis pubescens
Brassica tounefortii
Diplotaxis Cluster B
~20 M yrs
RAPA/ OLERACEA LINEAGE
NIGRA LINEAGE

Figure 1-1: The evolutionary heritage of the *Brassica* genomes according to RFLP analysis of chloroplast DNA (Warwick *et al.* 1992).

1.2. History and breeding of canola quality *Brassica napus*

Brassica napus (rapeseed) was originally cultivated by ancient civilizations in Asia and the Mediterranean. By 2000 BC, *B. napus* was being cultivated in India and by the 13th century, rapeseed was widely cultivated in Europe. The oil from rapeseed was used mainly as fuel in lamps, but was later used as cooking oil, and in some foods. During World War II, it was discovered that rapeseed oil would cling to water and steam washed metal surfaces better than other industrial oils. However, due to the war, by the early 1940's, European and Asian sources of rapeseed oil were being blocked. With the large demand for steam engine-powered naval and merchant ships, the demand for an appropriate lubricating oil increased. Prior to World War II, rapeseed had been grown in Canada in small research trials, which had indicated that rapeseed could be grown in northern latitudes; in cool temperatures and long days. In 1936, a farmer in Saskatchewan began to sell his seed to other producers. Due to the fact that this farmer was of Polish origin, this germplasm (which was *Brassica rapa* -formerly *Brassica campestris*) became referred to as "Polish rape". In 1943, increased demand for rapeseed resulted in Canada purchasing 19 tons of rapeseed from U.S. seed companies. This germplasm, which was originally from Argentina, proved to be *Brassica napus* and was subsequently referred to as "Argentine rapeseed" (*Canola council of Canada; www.canola-council.org*). By the end of World War II, the demand for rapeseed oil as a lubricant had decreased significantly and edible versions of *Brassica napus* (rapeseed) were being contemplated.

The fatty acid profile of any oil determines its use for edible or industrial uses. In the case of rapeseed, it contains significant amounts of eicosenoic, erucic acid and glucosinolates. Although erucic acid has not caused any documented cases of human toxicity, research has shown that high levels of erucic acid have been linked with increased fatty deposits in heart muscle in animals (*Canola council of Canada; www.canola-council.org*). By 1960, efforts had begun to breed low eicosenoic and erucic acid varieties, and by 1973, rapeseed oil had to contain less than 5% erucic acid. In addition to problems with erucic acid, rapeseed also contains glucosinolates, which are undesirable in cooking oils and in livestock feed, due to their sharp taste and anti-nutritive properties, but can be coveted for flavor in condiment mustard. In 1974, Dr. Baldur Stefansson, a University of Manitoba plant breeder, developed the first "double-low" variety, with reduced levels of both erucic acid and glucosinolates. This cultivar "Tower" was the first to be identified as 'canola' - an edible oil with nutritional requirements. Today, 'canola' is defined as any member of the Brassica that produces an oil that contains less than 2% erucic acid, and less than 30 micromoles per gram of air-dry, oil-free solid, of any one or any mixture of 3-butenyl glucosinolate, 4-pentenyl glucosinolate, 2-hydroxy-3 butenyl glucosinolate, and 2-hydroxy- 4-pentenyl glucosinolate (*Canola council of Canada; www.canola-council.org*). In Canada, a small amount of rapeseed is grown today, under contract, specifically for industrial uses as slip agents and in the production of plastics. However, rapeseed continues to be grown for edible cooking oil in many other countries (*Canola council of Canada; www.canola-council.org*).

Breeding in the 1970-1980's focused mainly on increasing the yield and oil content of canola crops. Breeders then focused on the concept of advancing sustainable farming practices and included goals such as drought tolerance, salinity tolerance, hybrid vigor, no-till farming practices, and disease/ pest resistance. Currently, the possible breeding of canola crops has expanded drastically with the routine genetic engineering of *Brassicas*. Transgenic applications currently include herbicide tolerance and hybrids, but biodegradable plastics, pharmaceuticals, phytoremediation applications, enhanced nutritive values, disease resistance, and pest resistance, are on the horizon. In addition, a number of specialty products such as ultra-low saturated fat and omega-3 enhanced canola oils are currently under development (Damude and Kinney 2007).

Most *Brassica napus* (canola) varieties are well adapted to the climate of western Canada as they are frost tolerant, and grow well in cool climates. Canola also provides farmers with the opportunity to employ diverse cropping rotations. As a broadleaf crop, canola offers an important disease break during the cultivation of monocot species (*Canola council of Canada; www.canola-council.org*). In addition, canola root exudates and root penetration effects have also been shown to have beneficial effects on later crops (Shabaz, 2006).

Prior to about 1975, the changes in *B. napus* cultivars were attained via traditional breeding, which included crossing outside a species or genera and mutational approaches. More recently, a number of other techniques have become available for breeding including tissue culture, doubled haploidy, the production of transgenics and molecular markers.

Canola yields are adversely affected by a variety of biotic factors including weeds, insects and diseases: viral, bacterial and fungal. Broad leaf weeds, especially weeds from the *Brassicaceae* family, are particular problems in canola crops, due to their close phylogenetic relationship. This problem has been significantly reduced since the advent of herbicide resistant cultivars such as Roundup-Ready, Pursuit SMART and Liberty Link canola (*Canola council of Canada; www.canola-council.org*).

Insect pests of canola include flea beetles (*Phyllotreta cruciferae)*, cut worms (*Agrotis infusa*), aphids (*Brevicorne brassicae* and *Lipaphis erysimi*), cabbage moths (*Plutella xylostella*) and a wide variety of other caterpillars, mites and beetles. Most viruses affecting canola crops (Beet western yellow virus, Cauliflower Mosiac virus) are also known to be transmitted by pests; adding to the importance of controlling their populations. The most significant biotic factor reducing canola yields are fungal pathogens; the most destructive of which has been the fungal disease 'blackleg' caused by the fungus *Leptosphaeria maculans* (*Canola council of Canada; www.canola-council.org*).

1.3. *Brassica carinata*

Brassica carinata (BBCC, where 2n=34, Figure 1-3) which is used as both a leaf vegetable and as an oilseed crop, originated from North-eastern Africa where its diploid parent species overlapped in the wild. *Brassica carinata* (also known as Ethiopian mustard or Abyssinian mustard) is a natural allopolyploid that behaves like a diploid during meiosis (Downey, 1989).

B. carinata possesses many desirable agronomic characteristics including resistance to heat and drought (Kumar *et al.,* 1984), pod shattering resistance (Alonso *et al.,* 1991), aluminum tolerance (Huang *et al.,* 2002) and tolerance to saline and late-sown conditions (Malik 1990). *Brassica carinata* is also resistant to some diseases that infect *B. napus* and *B. rapa* canola cultivars, such as blackleg disease, *Alternaria*, and Scelerotinia. *B. carinata* accessions are also prone to excessive vegetative growth, low oil content, high sulphur and compromised oil profiles (Getinet *et al.,* 1997). However, recently, high yielding, low erucic acid varieties have become available. In addition, the plant is amenable to microspore culture and has now been adapted to a shorter growing season.

1.4. Polyploidy in the *Brassica*

The joining of two diverged genomes into a common nucleus to establish a polyploid individual is arguably the single most important genetic mechanism in plant evolution and one that has affected the genomes of most angiosperms, and therefore most crop plants (reviewed by Rieseberg 1997). Domesticated and natural polyploids can be categorized into autopolyploids and allopolyploids.

Autopolyploids contain three or more sets of homologous chromosomes (AAAA) which are all derived from the same diploid progenitor species. Allopolyploids contain separate sets of non-homologous chromosomes derived from recent or ancient hybridizations between different species (AABB). Due to having separate sets of chromosomes, allopolyploids typically exhibit bivalent pairing and disomic inheritance (markers and genes segregate in a Mendelian fashion as two alleles), and even for mutational studies, they often behave as a diploid (G. Haughn, *pers. comm.*). Many crop genera such as; *Brassica, Coffea, Glycine, Oryza, Saccharum, Triticum,* and *Zea* are now believed to be or have been allopolyploids (Rieseberg *et al.* 2000).

Most flowering plants have undergone multiple polyploidization events in their past. Therefore, regions of collinearity and ancient associations to homeologous chromosomes of different species need to be considered when synthesizing, manipulating or breeding polyploids. Like many plants, the *Brassica* have large amounts of progenitor duplication within their genomes. The evolution of *Brassica* species indicate multiple events of genome polyploidization and recombination (Parkin *et al.*, 2002). Thus the *Brassica* are considered to be both ancient and current polyploids, and possess features attributable to both allo- and autopolyploids. *A. thaliana*, with a genome size of only 125 Mb (compared to ~650 Mb for *B. oleracea*), has been shown to be the result of a historical tetraploidization event occurring ~112 Myr ago (The *Arabidopsis* Initiative 2000). This tetraploidization occurred considerably before the estimated divergence of

Arabidopsis and *Brassica* between 14.5 and 20.4 Myr ago (Yang *et al.*, 1999). Subsequent to this tetraploidization event, a further triplication appears to have occurred within the *Brassica* lineage (Parkin *et al.*, 2002).

The six major modern *Brassica* species can themselves be divided into two categories based on their levels of polyploidization. Three species are diploid; *B. rapa* (AA), *B. nigra* (BB) and *B. oleracea* (CC). The other three are allopolyploids (amphidiploids), comprised of various pairings of the diploid genomes; *B. juncea* (AABB), *B. napus* (AACC) and *B. carinata* (BBCC) (U 1935; Figure 1-2). The component genomes in the amphidiploid species have been conserved with respect to their diploid ancestors (Lydiate *et al.*, 1993). Indeed, resynthesized *B. napus* lines, created from interspecific *B. rapa* X *B. oleracea* crosses, and natural *B. napus* accessions have been used as parents to create mapping populations (Parkin *et al.*, 1995). One of these was used in this thesis as a *B. napus* recurrent parent. Resynthesized *B. juncea* and *B. carinata* lines have also been created (Srivastava 2004; Jourdan 1993).

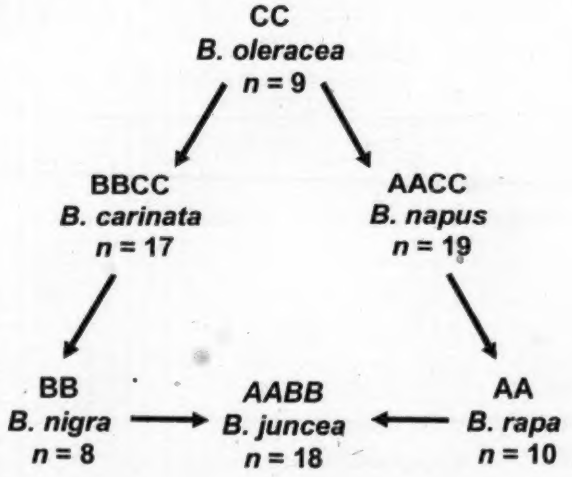


Figure 1-2: U's triangle. Genomic relation between diploid and amphidiploid *Brassica* species, redrawn from U (1935).

1.5. Blackleg disease in the *Brassica*

Leptosphaeria maculans, the causal agent of blackleg, is a filamentous ascomycete. Due to its impact on canola production, there is great interest in studying the biology of *L. maculans,* particularly factors contributing to infection and pathogenicity (Chen and Howlett, 1996). Other fungal diseases such as Sclerotinia stem rot (*Sclerotinia spp.*), Phytophthora root rot (*Phytophtohora megasperma*), downey mildew (*Peronospora parasitica*) and Alternaria leaf spot (*Alternaria brassicae*) are also significant diseases of *Brassicas (Canola council of Canada;* www.canola-council.org*)*.

L. maculans grows septate, branched, hyaline mycelia when young, but these turn darker with the buildup of pigment over time. This fungus produces wind borne ascospores after mating, which are thought to be the primary source of infection (Howlett *et al.*, 2001). Asexual pycnidiospores are also produced. These emerge in pinkish exudates and are likely spread to neighbouring plants through rain splash (Petrie 1978; Howlett *et al.*, 2001). *L. maculans* can survive saprophytically on stubble or crop residue, and may persist in field conditions for a few seasons in this manner. After spores are deposited on a plant, fungal hyphae germinate and extend across the surface, invading plant tissues through wounds or natural openings such as stomata (Hammond and Lewis, 1987a; Chen and Howlett, 1996). After entering the plant, the hyphae continue to grow, establishing small colonies within the mesophyll by four days after infection (Chen and Howlett, 1996).

Under susceptible conditions, hyphae growth continues biotrophically in the intercellular spaces of its host plant until approximately eight to ten days post infection. During this period few, if any, symptoms are visible. After this time, chlorotic patches and necrotic lesions begin to develop on infected plant tissues. Asexual reproduction, involving the production of fruiting bodies (pycnidia), often occurs within these necrotic lesions. These symptoms develop behind the growing hyphal front, and thereafter colonize the main vascular system of the plant (Hammond and Lewis, 1987a; 1987b). Growth proceeds in a biotrophic, symptomless manner until the hyphae reach the base of the stem, where it begins to invade and kill host cells, giving rise to the characteristic blackened-stem canker from which the disease name is derived (Hammond and Lewis, 1987b). This phase inhibits the transport of water and nutrients to the upper parts of the plant, and is responsible for the severe yield loss associated with blackleg. In advanced cases plants may collapse (lodge) and die before setting seed (*Canola council of Canada; www.canola-council.org*).

Initially reported in Saskatchewan in 1975, blackleg infestations in Canada are increasing due to the spread of virulent strains, coupled with an overall increase in canola cultivation (*Canola council of Canada; www.canola-council.org*). Blackleg can cause yield losses of up to an 80% in North America resulting in producers losing in excess of 50% of their gross revenue in infection zones. The traditional treatment of blackleg includes a variety of forms, including agricultural quarantine, crop rotations and the application of fungicides. Agricultural practices slow the advance of the disease into new areas, but do not control the disease in heavily infected areas and while fungicides have been moderately effective, they are expensive and detrimental to the environment. Thus, heritable resistance to blackleg is seen as the most effective means to control the disease (*Canola council of Canada; www.canola-council.org*).

1.6. Resistance to blackleg disease in the *Brassica*

Resistance to blackleg has been identified in several *B. napus* accessions; including French and Japanese varieties (Rimmer and Van Den Berg, 1992). Several researchers have described genetic mapping experiments to identify loci in the *B. napus* genome controlling resistance to blackleg. A resistance gene (*LEM1*) from the *B. napus* cultivar 'Major' was mapped to linkage group 6 by Ferreira *et al.* (1995). Several DNA (RFLP) markers linked to a gene, *LmFr1*, from the cultivar Crésor were identified by Dion *et al.* (1995). This gene was mapped to linkage group N7 (Rimmer *et al.*, 1999), which is equivalent to linkage group 6 of Ferreira *et al.* (1995). A resistance gene from the Australian cultivar Shiralee (designated *LmR1*) has also been mapped to linkage group N7 of *B. napus* (Mayerhofer *et al.*, 1997; Mayerhofer *et al.*, 2005). Furthermore, resistance genes in other *B. napus* cultivars, including Maluka, also map to the linkage group N7 (Rimmer *et al.*, 1999), suggesting that a single gene, or gene cluster, may be responsible for controlling resistance to blackleg in *B. napus* (Table 1-2). It is probable that the N7 gene (LmR1) is the same as *Rlm4*, which has been shown to be present in the French cultivar, Major (mapped to linkage group 6 by Ferreira *et al.*, 1995) and a number of Australian cultivars, including Maluka (Rouxel *et al.*, 2003). To date, no C genome loci conferring resistance to blackleg have been identified.

1.7. B genome resistance

Brassica species containing the B genome have high levels of resistance to blackleg (Rimmer and van den Berg 1992; Purwantara *et al.*, 1999). Various sources of *B. carinata* resistance have been examined (Pang and Halloran 1996; Plieske *et al.*, 1998; Dixelius, 1999). Numerous attempts have been made to transfer B-genome resistance into *B. napus* through interspecific hybridization and somatic hybridization from *B. nigra*, *B. juncea* and *B. carinata* (Chèvre *et al.* 1997, *B. juncea*; Pang and Halloran 1996, *B. juncea*; Roy 1984, *B. juncea*; Sacristan and Gerdemann 1986, *B. juncea* and *B. carinata*; Sjödin and Glimelius 1989, *B. juncea, B. nigra* and *B. carinata*). Keri *et al.* (1997) used classical genetic methods to identify two genes controlling resistance in *B. juncea*. Resistance genes from *B. nigra* and *B. juncea* have been assigned to linkage groups B4 and B8, respectively (Chèvre *et al.* 1996, 1997). The high resistance phenotype of the response associated with the B-genome resistance in *B. nigra*, *B. juncea*, and *B. carinata* is very similar (Chapter 3; Plieske *et al,*. 1998, Purwantara *et al.*, 1998, and Yu *et al.*, 2005)

Recently, B genome resistance has been identified in *B. juncea* and mapped to specific linkage groups on the B genome (Christianson *et al.*, 2006). What is unique about this study was that the mapping did not involve an interspecific cross, but rather used a rare susceptible genotype of *B. juncea*. Resistance to blackleg from *B. carinata* has yet to be mapped. Table 1-1 presents a current list of mapped loci conferring resistance to blackleg isolates.

Due to high collinearity found among the *Brassica* subgenomes, several researchers have initiated introgression projects which use breeding strategies that assume that B genome loci can be introgressed through homeologous recombination or translocation (Schelfhout *et al.*, 2006; Pang and Halloran 1996; Roy *et al.*, 1984). Observed deviations in expected introgression frequencies have often been attributed to environmental conditions (Plieske *et al.*, 1998). In addition, Lukens *et al.*, (2006) demonstrated that extensive alterations in DNA methylation occurred when isogenic resynthesized *B. napus* lines were created. These methylation changes were responsible for novel genetic diversity of the resynthesized lineages. Thus, novel polyploids and interspecific/intergeneric crosses could be accompanied by epigenetic changes rather than by genetic changes (Snowdon, 2007).

It is important to note that a B genome derived resistant *B. napus* cultivar has never been developed for licensed cultivation despite many efforts to do so (Plieske *et al.*, 1998; Dixelius 1999; Roussel *et al*,. 1999; Pang and Halloran 1996; Roy *et al.*, 1997; Roussel, 1999; Batra *et al.*, 1990; Brown *et al.*, 1997; Earle *et al.*, 1992; Gundimeda *et al.*, 1992; Landgren and Glimelius 1994; Li *et al.*, 1995; Nanda Kumar and Shivanna 1993; Sundberg 1998; and Glimelius 1991). Also, it is known that *L. maculans* can overcome major resistance genes and that single specific resistance genes do not provide a durable resistance (Sprague *et al.*, 2006; Brun *et al.*, 2000; Delourme *et al.*, 2004).

1.8. *Brassica* and collinearity with *Arabidopsis thaliana*

Arabidopsis thaliana molecular tools can be used to study various members of the *Brassica* group. *B. oleracea* (CC) and *A. thaliana* are thought to have diverged 15–20 million years ago (Figure 1-2) (Yang *et al.*, 1999). With the complete sequence now at hand, we know that of the 26,000 genes originally proposed, at least 70% have been duplicated. Local gene duplications and genome polyploidization processes have occurred in *Arabidopsis* and contributed to the observed repetitions. In all, there are fewer than 11,600 different genes, a figure that could be further depleted as researchers identify additional gene duplication (The *Arabidopsis* Initiative 2000).

Table 1-1: Loci conferring resistance to blackleg. The genome of mapped resistance genes are indicated in the left hand column (G). The next column represents the *Brassica* resistance trait donor. Donor lines were resistant to the *L. maculans* isolates indicated in the fourth column. Mapped location linkage groups and the publishing authors are indicated on the right (Delourme *et al.,* 2006).

G	LINE	Gene	Resistance to Isolate	Linkage group	Authors
A	Major	***LEM1 (Rlm4)***	PG2	N7 (lg6) LK10	Ferreira *et al.* 1995 (lg)
A	Cresor	***LmFr1 (LmR1)*** ***CLmR1, (aRLMc)***	Field population mix (Sasketchewan)	N7 (lg6) LK10	Dion *et al.* 1995, Mayerhofer *et al.* 2005, Rimmer, 2006.
A	Shiralee	***LmR1***	Canadian isolate mix	N7 (lg6) LK10	Mayerhofer *et al.* 1997(N),2005
A	Maluka	***CRLMm (Rlm4)***	PG2	N7 (lg6) LK10	Rimmer *et al.* 1999 (N), Rimmer, 2006.
A	*B. rapa*	***LepR1***	PG2, PG3, PG4,	N2	Yu *et al.* 2005 (N)
A	*B. rapa*	***LepR2***	PG2, PG3,partial PG4	N10	Yu *et al.* 2005
A	Jet Neuf	***Rlm1,3,4,7,9 In 35cM interval***	PG2	N7 (lg6) LK10	Delourme *et al.* 2004 (LK)
A	Quinta, Maxol	***Rlm1, Rlm4***	PG2, PG3, PG4	N7 (lg6) LK10	Ansan-Melayah *et al.*, 1998, Delourme *et al.*, 2004, Balesdent *et al.*, 2002.
A	Surpass 400	***LepR3***	PG2, PG3, PG4	LK10 (N7) lg6	Delourme *et al.* 2004 (LK)
A	Darmor	***Rlm9***	IBCN56	LK10 (N7) lg6	Delourme *et al.*, 2004, Balesdent *et al.*, 2002.
A	RB87-62	***aRLMrb***	PG2	N10 (20cM from LepR2)	Rimmer, 2006.
A	Glacier, Samourai	***Rlm2***	PG2	LK16	Rouxel *et al.* 2003, Delourme *et al.* 2004 (LK), Ansan-Melayah *et al.*, 1998
A	B. nigra			B4	Chevre *et al.* 1996
B	B. juncea			B8	Chevre *et al.* 1997
B	B. juncea	**RJlm2**			Saal *et al.* 2004
B	B. juncea	**LMJR1**	PG2, PG4	J13, G3	Christianson *et al.* 2005
B	B. juncea	**LMJR2**	PG2, PG4	J18	Christianson *et al.* 2005
B	*B. nirga*	***Lm1***		J13, J18Transgenic	Wretbblad *et al.* 2003

Analysis of the complete genome sequence of *A. thaliana* (The *Arabidopsis* Initiative 2000) revealed previously hidden duplications arising from as many as three rounds of polyploidization (Simillion *et al.*, 2002). Subsequent gene loss and genome rearrangements have resulted in its current arrangement of five chromosomes. The *Brassicas* have undergone further polyploidization events since the *Arabidopsis/Brassica* progenitor species of n=6, and these have resulted in more complex and duplicated genomes with n=8, 9 and 10. Grouped with *S. alba,* haploid chromosome numbers from n=7 to n=12 are found within the *Brassica* (Downey and Robbelen, 1989).

Large segments of collinearity have been demonstrated between *A. thaliana* and *B. napus* (Cavell *et al.*, 1998; Parkin *et al.*, 2002). Coding region sequences have been shown to be 87% similar between *A. thaliana* and *B. napus,* whereas in introns and intergenic regions, it is less than 70% (Cavell *et al.*, 1998; Quiros *et al.*, 2001). Genomic and EST sequences available for *A. thaliana* are useful in characterizing sequences from *Brassica* species. In particular, *A. thaliana* EST sequences with significant homology to disease resistance genes have been used as molecular markers to identify possible resistance gene candidates in *B. napus* where few resistance gene loci had previously been characterized (Sillito *et al.*, 2000). Despite being a close relative of the *Brassica*, *A. thaliana* is a non-host to the important *Brassica* pathogen *L. maculans*. Therefore, the interaction between *Brassica* and *L. maculans* can only be observed indirectly via *A. thaliana* genomic tools.

1.9. Mapping in polyploid crop species

Detecting duplicated regions in polyploids using linkage or sequence analyses is a non-trivial task given the occurrence of sequence repetition, chromosomal rearrangements and insertion–deletions altering chromosomal structure and gene complement (Mayerhofer *et al.* 2005; Milligan *et al.*, 1998). In addition, divergence in the nucleotide sequence of homeologous regions over long periods of time can obscure the shared ancestry of related chromosome segments. In linkage experiments, these difficulties are further compounded by incomplete marker polymorphism which can lead to underestimation of gene copy number and therefore the extent of gene duplication (Lukens *et al.*, 2004), as well as recombination rate abnormalities in altered chromosome regions (Mayerhofer *et al.*, 2005). Consequently, to make sound inferences regarding genome structure using genetic linkage data, it is essential to use dense linkage maps. Also, traditional assays for identifying BAC clones corresponding to a mapped DNA marker usually do not provide sufficient information to distinguish between allelic and non-allelic loci (Mayerhofer *et al.*, 2005).

The genetic history of a polyploid can also affect the interpretation of linkage data; as duplications, transposon insertions and recombination events do not occur equally in all parts of a plant genome. These genomic rearrangements can cause the recombination within an interval to become elevated or repressed relative to other intervals at other locations in the genome. For instance, in maize, large portions of the genome are composed of intergenic DNA, most of which is composed of retrotransposons inserted within each other. These elements show a distinct preference to be inserted into genes that have been duplicated within the last two to six million years (Brunner *et al.,* 2005). Hence, these elements account for a larger linkage distance between genes in this class, and larger linkage distances in the large genome grasses, which tend to be grasses that have undergone more recent polyploidization events.

Some plants have shown variable recombination rates according to the sex of the donor meiotic product. In some organisms, (i.e. Solanaceous species), recombination rates are higher in the female gametes. Conversely, rye, pine and maize show higher recombination rates when male gametes are observed (reviewed by Nelson *et al.,* 2005). For the *Brassica*, *B. oleracea* has been shown to have higher recombination rates in female gametes, compared to male meioses (Kearsey *et al.*, 1996). In *B. nigra*, higher male recombination frequencies were observed for terminal chromosomal regions, while higher female recombination frequencies were adjacent to putative centromeres (Lagercrantz and Lydiate, 1995). The related *Sinapis alba* has elevated recombination rates in pollen (Nelson *et al.,* 2005). The possibility that *B. carinata* and other *Brassica* species or hybrids show recombination distortion based on the sex of the parent has not been investigated. The contribution of gamete selection on recombination is difficult to define as age, variety and temperature can also alter these rates.

1.10. Mapping in *Brassica*

Using RFLP markers in a resynthesized *B. napus* population revealed that their inheritance was predominantly disomic (Parkin *et al.,* 1995). This indicated that the *B. rapa* derived chromosomes in the resynthesized parent were pairing exclusively with identifiable A genome homologues in *B. napus* during meiosis, although it has been shown in other crosses, that A/C homeologous recombination can also occur (Leflon *et al.*, 2006). Similarly, the *B. oleracea* derived chromosomes paired with C genome homologues (Parkin *et al.,* 1995). Furthermore, a genetic map of the *B. oleracea* genome was constructed using the same set of RFLP markers (Bohoun *et al.,* 1998). Strong collinearity was observed between the two C genome maps. This common set of RFLP markers has also been applied to the genomes of *B. juncea* (Axelsson *et al.*, 2000) and *B. nigra* (Lagercrantz and Lydiate, 1995). In the case of the *B. juncea* map, it was possible to differentiate between the A and B genome linkage groups and align them with their homologues from *B. nigra* and *B. napus* (Axelsson *et al.*, 2000). Marker based examinations of the ancestral A, B and C, genomes have demonstrated that although there were major chromosomal rearrangements between them, they each contain eight triplicated regions covering near the entirety of their genomes (Lagercrantz and Lydiate, 1996).

At present, a minimum of twenty molecular genetic maps have been constructed for the *Brassica*, including all of the major cultivated species, except for *Brassica carinata* (Choi *et al.*, 2007; Sun *et al.,* 2007; Piquemal *et al.*, 2005; Slocum *et al.,* 1990; Landry *et al.*, 1991, 1992; Song *et al.,* 1991; Chyi *et al.,* 1992; Kianian and Quiros, 1992; Figdore *et al.*, 1993; Ferreira *et al.,* 1994; Teutonico and Osborn, 1994; Uzunova *et al.,* 1995; Lagercrantz and Lydiate, 1996; Truco *et al.*, 1996; Cheung *et al.,* 1997, Axellson *et al.,* 2000, Christianson *et al.,* 2006 and Lan *et al.,* 2000a, 2000b). These linkage maps include at least 935 different publicly available probes, of which many have been included in multiple maps. Within each species, the maps show almost complete collinearity (Lydiate *et al.*, 1993), except for small inversions that differ among some *B. oleracea* genotypes (Kianian and Quiros, 1992, and Lan *et al.*, 2000a). It has been found that at least 22 chromosomal rearrangements differentiate *B. oleracea* homeologs from one another (Lan *et al.,* 2000a). Comparison of *B. rapa* with *B. oleracea* and *B. napus* supports the close evolutionary relationship between the two diploids (Figure 1-2) but indicates that deletions and insertions may have occurred after divergence of the two species (Hoenecke and Chyi, 1991). The genome of the synthetic *B. napus* varieties has remained essentially identical with respect to its *B. oleracea* and *B. rapa* progenitors

(Lydiate *et al.*, 1993), although the evolution of wild *B. napus* has been accompanied by additional rearrangements (Cheung and Landry, 1996). Extensive duplications of chromosome segments are observed in both the diploid and amphidiploid maps, supporting the hypothesis that diploid *Brassica spp.* are derived from an ancestor with a lower original basic chromosome number (Parkin *et al.* 2005). No duplications of whole linkage groups have been found.

1.11. Introgression and crop breeding

Introgression refers to the incorporation of genetic information from one species into another gene pool, or species, and plays a large role in the development of crops. Genetic information from outside groups can increase the heterogeneity of germplasm (the genetic variation within one interbreeding group or species) , and can incorporate particular traits for a specific disease or environmental factor. Although the process is valuable, introgression comes with its difficulties. Introgressions between species or genera often require tissue culture, techniques for chromosome doubling and additional crosses between buffering genome species to allow the cross to be viable. Also, hybridizing crosses often create transient allopolyploids which can have morphological anomalies, low fertility, unstable characteristics, and can introduce negative traits into desired germplasm. In addition to introgressing the desired trait, other features associated with initial polyploidy such as heterosis, and other genomic structural changes may also be of benefit and should be evaluated.

Possibly the most characterized example of a successful introgression has been the incorporation of the nematode resistance gene "*Mi*" into cultivated tomato (*Lycopersicon esculentum).* This gene has also been an excellent example of the use of host resistance to effectively reduce the need for pesticide application (Williamson 1998).

Mi resistance is used to describe plants that have a particular genetic resistance to infection from root-rot nematodes (*Meloidogyne* spp.). Root-knot nematodes are obligate, soil-dwelling, endoparasites that infect a large number of crop plants, cause severe yield loss, and can be difficult to control with any known agricultural chemical. The disease is characterized by the presence of galls or root-knots on infected plants. Non-resistant molecular reactions to nematode infection include those to wounding or stress caused by infection, but resistant plants perturb the initiation and maintenance of feeding sites. Plants are defined as resistant when they suppress nematode reproduction altogether (Williamson 1998).

Root-knot nematode resistance was originally identified in an accession of the wild tomato species, *Lycopersicon peruvianum*, a species that does not normally cross with cultivated tomato, *L. esculentum*. In the early 1940s, an interspecific hybrid was successfully produced using embryo rescue (Smith 1944). This introgressed resistance proved highly successful in protecting many varieties of tomato from nematode damage and allowed the tomato to be more widely cultivated. This one hybrid plant is the source of all root-knot nematode resistance in currently available fresh-market and processing tomato cultivars.

The *Mi* gene was isolated via positional cloning. The cloned *Mi* region, has allowed it to be transferred to tomato cultivars using marker-assisted selection and into other crop species, which did not have natural resistance (Milligan *et al.*, 1998).

Another example of introgression in the *Brassica* with significant commercial potential is the development of the cytoplasmic male sterility (CMS) system in *B. napus*. CMS is a maternally inherited trait that prevents the production of functional pollen but maintains female fertility and, together with nuclear restorer genes, is widely used for large-scale hybrid seed production in numerous crop species. Ogura CMS, and the corresponding nuclear restorer locus, *Rfo*, have been introgressed from radish *(Raphanus sativus)* into rapeseed (Heyn, 1976). However, as is often the case with intergeneric hybridizations, the introgression also introduced deleterious genetic characteristics from radish sequences remaining around the *Rfo* gene and elsewhere in the genome (Pellan-Delourme and Renard 1988). Extensive backcross and pedigree breeding were necessary to improve the low female fertility of the restorer lines and high glucosinolate content, but with limited success, due to tight linkage and impaired meiotic recombination between radish and rapeseed DNA. The *Rfo* gene was localized on a *B. napus* genetic map and it was determined that the introgression had taken place in the C genome of rapeseed through homoeologous recombination, replacing a non-distal *B. napus* region of around 50 cM (Delourme *et al.*,1995, 1998). Due to these difficulties in the development of agronomically useful *Rfo* restorer lines by traditional breeding methods, programs were initiated to isolate the *Rfo* gene from radish via a positional cloning approach. Two groups, using radish mapping populations segregating for the *Rfo* gene, exploited *Raphanus-Arabidopsis* microsynteny in that region and genetically and physically delimited the locus. The *Rfo* gene was cloned independently and shown to be a member of the pentatricopeptide-repeat protein family (Brown *et al.*, 2003; Desloire *et al.*, 2003). Transformed *B. napus* plants, carrying the cloned *Rfo* gene from radish, were not noticeably different from fertile rapeseed plants and did not have elevated levels of glucosinolates, indicating that the transgenic approach is likely to be successful for the commercial exploitation of the Ogura CMS/*Rfo* two-component system (Brown et al. 2003). This example illustrates the challenges such as linkage drag, reduced recombination rates and complicated mapping analysis that are involved in introgessing genes of interest from different species. It also demonstrates the need for detailed mapping analyses of introgression events in the amphidiploid Brassicas before any synteny with Arabidopsis can be exploited.

1.12. Research hypotheses and objectives

Until recently, it was difficult to conduct the detailed genetics and mapping studies that are required to map and clone genes from agronomically important crop plants. However, with the development of molecular markers, sequence information and detailed genetic maps, it is now becoming possible to begin to address these questions. For example, the late Charlie Rich (UC, Davis) and Steven Tanksley's group (Cornell) have developed tomato genetic resources which are resulting in the cloning of a number of agronomically important QTL's in tomato (Martin *et al.* 1993; Frary *et al.* 2000). Hence, one of the keys to detailed mapping studies is the availability of genetic markers.

As genomic and cDNA sequencing projects progress, a tremendous amount of sequence information is becoming publicly available. These sequence resources have been exploited for gene discovery and marker development. Simple sequence repeat (SSR) markers are among the most useful because of their great variability, abundance, and ease of analysis. This research makes use of a bank of approximately 3000 characterized SSR markers (primer-pairs) identifying loci on the A, B and C *Brassica* genomes. The majority of these markers are proprietary; however care was taken to include a subset of publicly available SSR markers on each linkage group. In addition, AAFC (Agriculture and Agri-Food Canada Saskatoon) has characterized these SSR markers using three intraspecific maps which will be used to align and corroborate the molecular maps described in this thesis. These are the 61-9 and 72-8 *B. napus* maps and the *B. juncea* T-1 map (Parkin *et al,* 1995; Axelsson *et al.,* 2000).

The objectives of this research included the following. First, I was interested in using *B. carinata* as a source of genetic traits to introgress into *B. napus*. To date, *B. carinata* has not been used as a source of traits, where the introgression could be tracked using molecular markers. In order to achieve this, my first objective was to generate a set of backcross populations that could be used in generating a linkage map for the C and B chromosomes of the inter-specific germplasm. The generation of these linkage maps then allowed me to determine the recombination rates and alterations in genetic architecture that occur in the inter-specific crosses between *B. carinata* and *B. napus*.

Some of the specific hypotheses that were tested using the novel inter-specific lineages included the following: Could interspecific crosses between *B. carinata* and *B. napus* be created? If so, could B genome traits (i.e. blackleg resistance) be transferred from the B genome to the A/C genomes, and if not, could the resistance be attributed to a B genome chromosome using molecular markers? If previously unreported genetic phenomenon occur in these crosses, I wanted to define what they were (recombination rates, segregation distortion and changes in genetic architecture), and how these changes would affect *Brassica* interspecific crosses in general. I also wanted to evaluate the effect of different parental germplasm, reciprocal crosses, and progeny fertility on these phenomena.

One of the key traits of interest on the B genome is blackleg resistance, due to the fact that B genome containing species have high levels of resistance (Pang and

Halloran 1996; Chevre *et al.*, 1997; Keri *et al.* 1997; Dixelius 1999; Dixelius and Wahlberg 1999; Plieske and Struss 2001; Wretblad *et al.*, 2003). As mentioned previously, to our knowledge B genome resistance has not been transferred to a canola cultivar. Projects that have attempted these introgressions have been numerous, have been repeated, and are ongoing. Given the amount of resources required to complete these types of investigations, and the lack of success thus far, it is important to determine why these types of projects have failed. This thesis tracks the genetic changes in *B. napus*/ *B. carinata* interspecific hybrid genomes, and I provide an analysis of the issues associated with the failure of most B genome introgressions.

The methods and resources used to complete this project are described in Chapter 2. The breeding and development of the hybrid lineages, observed fertility and selection of these interspecific lineages is described in detail in Chapter 3. Chapters 4 and 5 describe the introgression of the *B. carinata* genome through four generations of backcross breeding. Chapter 4 describes the observed *B. carinata* introgressions in a BC1 mapping population (KCN-5 mapping population) through the use of SSR markers and presents linkage maps of the C and B genome chromosomes involved in the cross. Chapter 5 extends these observations to include more advanced backcross germplasm and tracks *B. carinata* introgressions through to a BC3 stage of breeding (KCN-10 mapping population). In both cases, linkage maps are used to describe introgressions and recombination frequencies (RFs) apparent in the germplasm. Finally, Chapter 6 provides an overview and discussion of some of the key findings in this thesis and discusses how this type of approach can be used to successfully introgress traits of interest into crop plants.

2. Materials and Methods

2.1. Plant materials and mapping populations

Population development was difficult due to the interspecific nature and inherent incompatibility mechanisms associated with these crosses. In addition, *B. carinata* takes nine months to mature in the greenhouse. The population development described below took four years, between 1999 and 2004.

Brassica carinata lines were chosen based on blackleg disease ratings provided by Purwantara *et al.* (1998). Two *B. carinata* lines were shown to be highly resistant to five virulent Australian *Leptosphaeria maculans* isolates (Purwantara *et al.,* 1998). Two plants, (renamed *BCA-070* and *BCA-065* from the 'BJU-' prefix), were used as the resistant *B. carinata* parents. Both *B.carinata* accessions were grown in 1999 and allowed to self-seed once to promote homozygosity of the seed source prior to interspecific crossing.

The species designation (*B. carinata*) of *BCA-070* and *BCA-065* were confirmed through cytology. Flower buds were harvested and immediately fixed in 3:1, 70% ethanol/glacial acetic acid and then stored at 4°C. Anthers at the metaphase 1 stage of meiosis were squashed and stained with a drop of 1% acetocarmine solution and nuclei were studied by phase contrast microscopy (Belling, 1926).

B. napus lines were chosen based on susceptibility to *L. maculans*. *MBX-2* (A12) is a resynthesized *B. napus* developed at the Agriculture and Agri-Food Canada Saskatoon Research Center (AAFC Saskatoon) (Parkin *et al.,* 1995). *B. napus cv* Topas is a publicly available blackleg susceptible canola quality cultivar which was chosen because of its ability to produce doubled haploid (DH) offspring. Up to 50% of cultured Topas microspores have been reported to undergo embryogenesis under optimal conditions (Pechan and Keller, 1988).

N-o-1 is a doubled haploid line derived from the cultivar Westar, which is a standard Canadian canola-quality summer rape variety developed in the 1970's. N-o-1 was initially used as a *B. napus* parent, but no surviving ovules were generated between the interspecific crosses (Chapter 3). Westar has been used extensively in research, is easily transformed, and is well characterized (Sharpe *et. al.* 1995, Osborne *et. al.* 2003). DH12075 is a double-haploid *B. napus* line derived from a cross between Crésor and Westar, (Li *et al.*, 2003) which was used in backcrosses to F1 plants with the goal of providing a potentially polymorphic reference point to generate a linkage map of the A genome. Plants that were derived from a backcross with DH12075, instead of their recurrent parent, were denoted with 'HC' (horizontal cross) in their designations (i.e.KCN-5-HC-10). These crosses were not used in the study.

B. carinata and *B. napus* parents were crossed reciprocally in all combinations via bud-pollination. Buds of a single plant were used for each reciprocal cross, such that individual plants of *BCA-070, BCA-065, Topas* and *MBX2* were used as the maternal and paternal parents. Buds from female donors were emasculated with sterile tweezers just before opening. Pollen from the male donor was deposited on the mature stigmas of the receptive plants and the flower was bagged to prevent further pollination. Immature stigmas and open buds were discarded. After pollination, three-week old siliques were removed from the plant, and sterilized in 10% bleach for 1 minute. The ovules were excised from the siliques and cultured on modified Murashige and Skoog (MS) media containing 300mg/L casein hydrolysate, 2.5g/L gelrite, and 50g/ L sucrose (Murashige and Skoog 1962). Ovules were transferred to fresh modified-MS media once a month, or as needed. Surviving ovules were cultured until germination or callus formation, and were subsequently transferred to modified-MS media containing 100 μL of a 0.15mg/mL solution of naphthaleneacetic acid (NAA). Well-rooted plantlets were then transferred to soil-less media (280 MetroMix, Sungro, Vancouver, B.C.). Ten hybrid plants (named KCN-1 to KCN-10) were generated in this manner (Chapter 3).

Hybrid plants were backcrossed with their recurrent *B. napus* parent clone to yield ten families of BC1 seed (described in Chapter 3). From these ten families, 210 BC1 plants were grown for genomic DNA extraction, selfed seed (BC1F2) and to propagate BC2 seed. Some BC2 seedlings and BC1F2 seedlings from these families were screened for blackleg resistance to select a BC1 mapping population and another resistant BC1 lineage for further mapping population development. Several families were continued in this manner to the BC3 generation, but two lineages were selected for population development (Figure 2-1 and 2-2). These two lineages were derived from the F1 hybrids KCN-5 and KCN-10. Both F1 plants (KCN-5 and KCN-10), both mapping populations derived from these plants and three Sibling families (KCN-5 BC2, KCN-10 BC1 and KCN-10 BC2) were characterized with molecular markers. Although the Sibling families were small, they could be assayed for the presence or absence of known loci and they provided an illustration of the loss of *B. carinata* loci through successive generations.

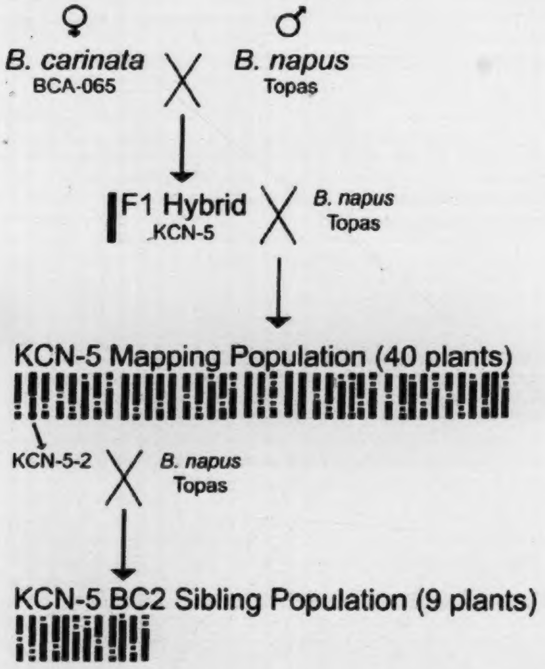

♀
B. carinata
BCA-065
♂
B. napus
Topas
F1 Hybrid
KCN-5
B. napus
Topas
KCN-5 Mapping Population (40 plants)
KCN-5-2
B. napus
Topas
KCN-5 BC2 Sibling Population (9 plants)

Figure 2-1: KCN-5 Mapping Population development. A BC1 mapping population of 40 individuals was derived from a cross between BCA-065 (*B. carinata)* and Topas (*B. napus*). The F1 and subsequent generations were backcrossed onto the *B. napus* recurrent parent clone. The KCN-5-2 plant was the female progenitor to the KCN-5 BC2 Sibling family.

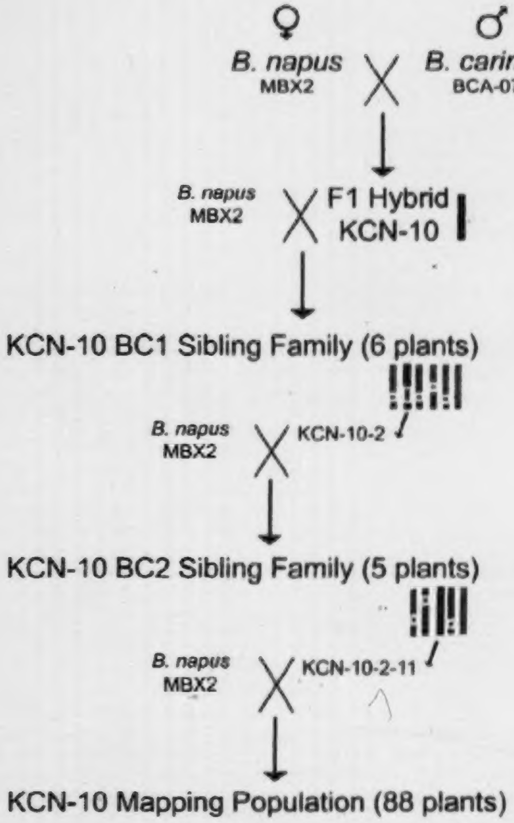

♀
♂
B. napus
MBX2
B. carinata
BCA-070
B. napus
MBX2
F1 Hybrid
KCN-10
KCN-10 BC1 Sibling Family (6 plants)
B. napus
MBX2
KCN-10-2
KCN-10 BC2 Sibling Family (5 plants)
B. napus
MBX2
KCN-10-2-11
KCN-10 Mapping Population (88 plants)

Figure 2-2: KCN-10 Mapping Population development. A BC3 mapping population of 88 plants was derived from a cross between BCA-070 (*B. carinata)* and MBX-2 (*B. napus*). The F1 and subsequent generations were backcrossed to the *B. napus* recurrent parent clone. The BC1 KCN-10-2 plant was the female progenitor to the KCN-10 BC2 Sibling family and KCN-10-2-11 was the female progenitor to the KCN-10 Mapping Population.

2.2. Stem section tissue culture

To ensure the genetic uniformity of the recurrent parents, all backcrosses used pollen from regenerated *B. napus* clones derived from the original MBX-2 or Topas plant. Six cm stem cuttings were taken from the original *B. napus* parent, swabbed in 70% ethanol, and then soaked in a 10% bleach solution for 6 minutes. Stems were rinsed in distilled sterile water four times for 6 minutes each. The stems were then sliced into 5 mm sections and plated on MS media supplemented with 3% sucrose. After two weeks of culture, stem segments were transferred to MS media containing 100μL of a surface spread 0.15μg/ ml solution of napthalene acetic acid (NAA) for 2 weeks. After the appearance of roots, the tissue was transferred to MS containing 100μl of a surface spread 0.3mg/mL solution of benzol aminopurine (BA). Plantlets with both roots and shoots were then moved to soil-less potting media (Redi-Earth, SunGro, Vancouver, B.C).

2.3. Growth conditions and seed storage

Seed for this project was dried and stored at 4°C. Seeds were subsequently germinated on damp, acid purified sand, (S0124, Sigma, St. Louis, MO). If seed appeared small or desiccated, 10 μM gibberellic acid (GA3) in H_2O was used to promote germination. After germination, seedlings were transferred to 6 inch pots containing 280 Metro-Mix soil-less potting mixture (SunGro, Vancouver, B.C). Growing plants were watered every other day as the plants matured, and were fertilized with a 20N-20P-20K solution every 10-14 days. Plants were grown with a 16-hour photoperiod with day/night temperatures of 16-18/10-12°C, respectively.

2.4. *L. maculans* resistance scoring of cotyledons

It should be noted that parental germplasm was screened to ensure that *B. carinata* lines were blackleg resistant and that all *B. napus* lines were susceptible. Progeny scoring was conducted for several hybrid individuals to verify that this trait was being conserved though successive generations, but blackleg ratings were not used to make selections for population development. This was because all F1s, and almost all BC1s and BC2s screened were resistant. Primarily, the main focus of the research shifted towards answering questions concerning chromosome introgression. The mapping population progenitors KCN-5 and KCN-10-2-11, were subsequently shown to be blackleg resistant.

Two different protocols were used for determining blackleg resistance. For parental, BC1, BC1F2 and BC2 disease ratings, plants were screened using the standard protocol from the canola mapping group at the University of Alberta. This method used multiple *L. maculans* isolates and is described later in this section. The KCN-10 mapping population was scored in Saskatoon, SK at the AAFC research station using single *L. maculans* isolates. The parental lines were also re-screened using this method. The single isolate method is favored amongst plant pathologists as it allows the researcher to discriminate between different cotyledon reactions to the different isolates. The multiple isolate method is preferred amongst plant breeders as it more closely represents the natural pathogen flora found in the field.

L. maculans isolates WA74 and WA51 were collected in Western Australia, and rated as virulent isolates PG2 and PG4, respectively (Mengistu *et al.*, 1991). These isolates are maintained in the collection at the AAFC Saskatoon Research Center. Inoculum was prepared by culturing Westar cotyledons infected with individual isolates of *L. maculans* on V-8 agar plates (20% Campbells V-8 juice, 0.075% calcium carbonate and 100μg/l streptomycin sulfate). Detached cotyledons were sterilized in 12% sodium hypochlorite for 2 min and then washed in sterile water before plating. Plates were incubated at 22°C under continuous fluorescent light. Approximately 2 weeks later, spores were collected by applying 10 ml sterile water to the plate surface and scraping off the submerged pycnidia. The resulting spore suspension was filtered through a double layer of cheesecloth to remove pycnidial remnants, and the spore concentration was adjusted to 2×10^7 spores ml^{-1}, using a hemacytometer. Spore suspensions were stored at –20°C and thawed just before inoculation (Yu *et al.*, 2005).

For growth chamber evaluations of cotyledon blackleg resistance, a minimum of 16 F2 plants per progenitor plant were germinated in 96-well flats. Soil-less media composed of peat moss, sand, medium grade vermiculite, and fine ground calcium carbonate was used as the growth medium. Seedlings were germinated in growth chambers and watered daily via bottom capillary action. The plants were grown under conditions of a 20/18°C day/night temperature and a 16 hour photoperiod. Grow lights were placed 12 inches above the seeding flats to encourage short hypocotyl development (Yu *et al.*, 2005).

Prior to the emergence of the first true leaves, cotyledons were inoculated by puncturing each lobe with a 21 gauge needle (4 punctures/plant, two/cotyledon), and a 10μL droplet of the WA74 pycnidiospore suspension was pipetted on the wounds of one cotyledon, while the other cotyledon of the same plant was inoculated with WA51 (Yu *et al.*, 2005).

Inoculated cotyledons were allowed to dry for at least 24 h before the inoculated seedlings were watered. In most cases, emerging leaves were removed to enhance cotyledon retention. Disease reaction was rated 10 days after inoculation, using the 0–9 scale described in Delwiche (1980). *B. napus* cultivars "Quinta", "Westar" and "Glacier" were used as controls for the disease ratings (Mengistu *et al.*, 1991). Disease ratings of 0 indicated a hypersensitive response, 1–6 described resistant interactions, while ratings of 7–9 described susceptible interactions (Delwiche, 1980). After rating, some highly resistant plants (ratings of 0-1) were repotted into 4 inch pots for tissue and seed collection.

For BC1, BC1F2 and BC2 disease ratings, cotyledon resistance responses to blackleg were determined as described by Mayerhofer *et al.* (1997). This method (multiple isolate method) employed multiple *L. maculans* isolates from the Canadian prairies, which were deposited onto the same 12 gauge needle wound in each cotyledon. The resistance phenotype of each backcross line was determined using a minimum of 16 seedlings from each lineage of interest. Plants were scored on a 1-4 scale, with 1 being resistant and 4 being highly susceptible. The publicly available cultivars "Westar", "Topas", and "Quantum" were used as control lines. Plants were then rescued from further infection by removing cotyledons following the 14 day scoring period. Some susceptible plants were rescued by culturing a meristem explant on soil-less media after dipping the base in 0.3% indole butyric acid (IBA) rooting powder (GardenTech, Lexington, KY).

2.5. Genomic DNA extractions, Southern hybridizations and probing with restriction fragment length polymorphism (RFLP) markers

Genomic DNA extractions and Southern hybridizations were performed as described by Sharpe *et al.*, (1995). Fifteen μg's of genomic DNA from each sample was digested with one unit of *Eco*RI. Digested samples were separated on agarose gels. The gels were blotted using a standard alkali transfer protocol onto Hybond N nylon membranes (Amersham Biosciences, Piscataway, NJ). Southern blots of genomic DNA were probed with a set of P^{32} labeled RFLP markers known to detect RFLPs in *Brassica* species (Sharpe *et al.*, 1995; Axelsson *et al.*, 2000, Mayerhofer *et al.*, 2005).

2.6. SSR markers

SSR primer pairs, derived from *B. nigra*, *B. juncea*, *B .rapa* and *B. napus*, with defined loci in the *Brassica* A, C and B genomes, were developed by Agriculture and Agri-Food Canada (AAFC), Saskatoon Research Station (Available on request under Material Transfer Agreement; http://www.agr.gc.ca). From these, a subset of markers that identified polymorphic loci on the *Brassica* C and B genomes were used to characterize the two hybrid families and their respective mapping populations. Amplification of microsatellite alleles by PCR was performed using an ABI 877 thermocycler/liquid handling robot (Applied Biosystems Inc., Foster City, CA) or the MWG Primus HT multiblock thermocycler (MWG Biotech). Microsatellite regions were amplified using specific paired primers (0.33 μM), one of which was labeled with a fluorescent dye (Hex (yellow), Fam (blue), or Tet (green); Applied Biosystems Inc. Foster City, CA). Amplification reactions were carried out with 40ng of genomic DNA, 0.2 mM dNTPs, 2.5mM $MgCl_2$, and 0.5U Amplitaq Gold (Applied Biosystems Inc. Foster City, CA) under the following conditions: 95°C for 10 min, seven cycles of 94°C for 15s; 50°C for 15s and 72°C for 30s; 27 cycles of 89°C for 15s; 50°C for 15s and 72°C for 30s and a final extension step of 72°C for 10 min. Each SSR marker was amplified separately and then three reactions were then pooled (each using a different dye) and separated through polyacrylamide gels on an ABI 377 automated DNA sequencer (Applied Biosystems Inc., Foster City, CA). Amplification reactions were carried out as described by Naom *et al.*, (1995).

Table 2-1 indicates the SSR markers that were mapped to specific linkage groups in the mapping populations. The primer sequences are available through AAFC, Saskatoon Research Station, under a material transfer agreement (D.J. Lydiate and A.G. Sharpe; AAFC Saskatoon Research Centre, Saskatoon, SK; http://www.agr.gc.ca).

Table 2-1: The complete list of SSR primer pairs that amplified one or more informative loci in one or more of the mapping populations.

SSR Markers						
sB0426	sB1871	sJ0397R	sN0412	sN13039	sN9292	sORG95
sB0744i	sB1935	sJ0502	sN0464	sN13083	sN9425	sORh13
sB0202i	sB1936	sJ0644	sN0583	sN1838	sN9427	sORh24
sB02124	sB1956	sJ0655	sN0584	sN1844	sN9431	sORh28
sB0268	sB1992	sJ0927	sN0704	sN1925	sN9542	sORh43
sB0273	sB2108F	sJ1071	sN0744E	sN1958	sN9756	sR12095
sB0334	sB2120	sJ1086	sN0758	sN1988	sN9875	sR1210
sB0360	sB2303	sJ1102	sN0768	sN2034	sNRB35	sR1243
sB0372	sB23194	sJ1158	sN0818F	sN2044	sNRB45	sR1346
sB0563i	sB2334	sJ13101	sN0866	sN2305	sNRD40	sR2028
sB0570	sB2352F	sJ13133	sN0983F	sN2429	sNRE30	sR2319
sB0580R	sB2365	sJ1322	sN10718	sN2440	sNRE46	sR6083
sB0860	sB2510	sJ1325i	sN11516	sN2443	sNRE47	sR6212
sB0862	sB2543R	sJ1473	sN11555	sN2834	sNRE85	sR7178
sB1060F	sB2545	sJ1505	sN11670	sN3508F	sNRh63	sR9477
sB1060Fi	sB2596	sJ1536	sN11710	sN3565R	sORC36	sS1717
sB1538	sB2668	sJ1822	sN11859	sN3676	sORC20	sS1852
sB1726	sB2755	sJ2013	sN11864	sN3761	sORC21	sS1907
sB1728	sB2769	sJ2042	sN11910	sN3828	sORC76	sS1949
sB1729	sB2771	sJ3302Ri	sN12118	sN3888F	sORC88	sS2076
sB1752	sB2972ii	sJ3327R	sN12480	sN3916	sORC95	sS2082
sB1755	sB3103	sJ3503R	sN12508ii	sN3922	sORD25	sS2136
sB1768	sB31111	sJ3838F	sN12574	sN4276	sORE30	sS2206
sB1772i	sB3176F	sJ3910	sN12624	sN4513F	sORE37	sS2210
sB1789	sB3910	sJ39119i	sN12624	sN5088F	sORE79	sS2225
sB1817	sB4727F	sJ3969	sN12670	sN7518	sORE94	sS2268
sB1828	sJ0143	sJ46102	sN12708	sN8115F	sORF31	sS2277
sB1839	sJ0266	sJ4633	sN12766	sN8348	sORF37	sS2331
sB1856	sJ03110	sN0229	sN12917	sN8841	sORF89	sS2368
sB1859	sJ03132	sN0406	sN12963	sN8956R	sORG90	

2.7. Map construction and linkage distance calculations

SSR markers that generated one or more polymorphic alleles were scored to generate scoring matrices (Appendix A). The presence of a *B. carinata* allele was designated as a plus (+), while the absence of a *B. carinata* allele was designated by a minus (-). The zero (0) designation indicated a failed PCR reaction. A 'minus' scoring was assigned if at least one other allele was amplified by the same marker in a particular genomic DNA lane. This could be distinguished from a failed PCR reaction in which no discernable bands were amplified in a particular lane. Exceptions to these assignments are discussed in Chapter 4. The assignment of loci onto chromosomes was accomplished by way of allele identity (discussed in Chapter 4) and linkage analysis.

Genetic linkage analysis was performed using MAPMAKER 3.0 (Lander *et al.,* 1987). A minimum LOD score of 4.0 and a maximum distance of 50.0 cM were used for the initial linkage association. A LOD score of 3.0 was used to bridge any larger gaps. Ordering markers within linkage groups was performed using the order command 7 of MAPMAKER. Scorings were reconfirmed in instances where double crossovers were observed on linkage groups N11, N13, and J11 - J18. Recombination frequencies were converted to Kosambi centiMorgan map distances (Kosambi, 1944).

Following initial alignments, the linkage analysis was completed using a LOD of 3.0, the 'ripple' and 'automap' mapping functions of the MapDisto 1.2.0.3 software package (Lorieux, 2002). MapDisto is a user-friendly Excel (Microsoft, Redmond, WA) based software program that generates graphical linkage groups, and has flipping functions that can be used to find optimal linkage group segment orientations.

Segregation distortion for the mapping populations were verified with Chi-squared tests contrasting expected 1:1 allele segregation with the values observed. The following equation was used:

$$\chi^2 = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i}$$

where O_i = the observed numbers; E_i = the expected numbers, assuming the 1:1 null (Ho) hypothesis (Johnson and Bhattacharyya, 1996).

2.8. Graphical genotypes

Graphical genotypes, first developed by Young and Tanksley (1989) are similar to karyotypes in describing a single chromosome in a single image, but differ in that the genomic constitution and parental derivation are indicated (Rieseberg *et al.*, 2000). In this thesis, 151 *B. carinata/B. napus* backcross plants were characterized via graphical genotypes. Graphical genotype diagrams were constructed such that if two adjacent markers were from the same parent, then the intervening block was assumed to be from that parent. Conversely, if adjacent markers differed in parental origin, then the intervening block was assumed to contain a crossover event. The graphical genotype was then constructed by minimizing the total number of crossover events required to explain a given genotype. The probability of multiple crossovers between two markers (which would be undetectable) was deemed low for early generation hybrids, and for small marker distance intervals (Rieseberg *et al.*, 2003).

Linkage groups were aligned according to SSR scoring matrix data (Appendix A). When SSR scoring data was insufficient to determine the marker order due to a lack of recombination (i.e. B genome linkage groups), reference mapping populations were used to determine the marker alignment (AAFC reference maps). The AAFC reference maps consisted of the 61-9/72-8 and T-1 mapping populations. The 61-9 population of doubled haploid (DH) lines was derived from a resynthesized *B. napus* crossed to a *B. napus* winter double low oilseed rape (Parkin *et al.*, 1995; Parkin *et al.*, 2005). The 72-8 population is a set of DH lines derived from a winter double low oilseed *B. napus* and a spring *B. napus* cross (Sharpe *et al.*, 1995; Parkin and Lydiate, 1997). The *B. juncea* T-1 population is a population of backcross individuals resulting from crossing a resynthesized *B. juncea* with two natural *B. juncea* lines, and is described in detail by Axelsson *et al.* (2000). The AAFC *Brassica* microsatellite consortia for *B. napus* and *B. juncea* microsatellite development used the 61-9, 72-8 and T-1 populations, respectively, to map SSR loci in the two species. The SSR primer-pair sequences mapped to the 61-9 and 72-8 populations were derived from *B. napus, B. rapa,* and *B. oleracea.* The primer pair sequences mapping to the T-1 population were derived from *B. nigra* and *B. juncea* germplasm (proprietary, Lydiate, D. and Sharpe, A. *pers. comm.*). The T-1 map was used to provide information in regard to the B genome, while the 61-9 and 72-8 populations provided information in regard to the C genome (proprietary, Lydiate, D. and Sharpe, A. *pers. comm.*).

2.9. Comparing recombination frequencies between the AAFC reference maps and the KCN-5 mapping population

Burke *et al.* (2004), when comparing RF values in *Helianthus* maps, determined the distance between the outermost shared markers on each linkage group between two maps. While this method can provide a general sense of recombination between two populations, it cannot account for different numbers and locations of markers between populations, which can change the perceived RF values of a population.

In order to compare the linkage groups characterized in this study with those previously characterized by AAFC Saskatoon, identical SSR marker loci were used to recalculate recombination distances in the AAFC reference maps, between markers on each chromosome. This was necessary because the AAFC reference population included additional markers, which would result in increased map distances between markers. The recalculation was done by reducing the AAFC reference maps and the KCN-5 mapping population scoring matrices to common loci. The linkage analysis was then completed using a LOD of 3.0, the 'ripple' and 'automap' mapping functions of the MapDisto 1.2.0.3 software package (Lorieux, 2002). The reduced KCN-5 mapping population linkage map was then contrasted with the corresponding reduced C or B genome AAFC reference maps (Parkin *et al.*, 1995, Parkin *et al.*, 2005, Sharpe *et al.*, 1995, Parkin and Lydiate, 1997, and Axelsson *et al.*, 2000). To verify that the RF values between the reduced KCN-5 mapping population matrix and the reduced AAFC reference population matrix were different, statistical significance of the difference between the RF values was determined by using a standard two-proportion Z-test for proportions with unequal variances (95% confidence interval). This statistical test was used because the proportional data was not discrete (required for chi-squared tests) and the Z-test allows for unequal variances in the data sets.

$$z = \frac{(\hat{p}_1 - \hat{p}_2) - (p_1 - p_2)}{\sqrt{\frac{\hat{p}_1(1-\hat{p}_1)}{n_1} + \frac{\hat{p}_2(1-\hat{p}_2)}{n_2}}}$$

Where n= population size, and p= proportion (RF), $n1p1 > 5$ and $n1\ (1 - p1) > 5$ and $n2p2 > 5$ and $n2\ (1 - p2) > 5$ and independent observations (Johnson and Bhattacharyya, 1996).

3. Phenotypic evaluation of two *B. napus/B. carinata* interspecific populations

The main objective of this study was to monitor the introgression of the *B. carinata* genome into a *B. napus* background using molecular markers. In order to develop the germplasm for this study, interspecific families of *B. carinata/B. napus* germplasm were produced (described in Chapter 2). Although blackleg resistance did not continue to be the most significant aspect of this research, the introgression scheme was initially modeled on a typical blackleg introgression program. Therefore, it was important that the *B. carinata* donor lines expressed blackleg resistance and conversely, that the *B. napus* lines were susceptible. Additionally, the *B. napus* lines needed to exhibit canola quality oil profiles and ideally be conducive to tissue culture.

Following the selection of parental lines, reciprocal interspecific crosses were performed to generate ten F1 hybrids via ovule rescue tissue culture. A series of backcrosses were performed on each F1 hybrid individual, with *B. napus* as the recurrent parent. Using blackleg screening and fertility as the selection criteria, two blackleg resistant lineages were chosen to be the focus of this investigation. These were termed the KCN-5 and KCN-10 mapping populations (Figure 2-1 and 2-2 respectively). The KCN-5 mapping population was developed directly from the F1 KCN-5 plant and was composed of 40 BC1 plants. The KCN-10 mapping population was developed directly from the BC2 plant KCN-10-2-11, and was a family of 88 BC3 individuals. This chapter presents data on the seed production, germination rates and blackleg resistance of the early-stage interspecific germplasm.

3.1. Blackleg resistance of parental germplasm

The *Brassica* parents used in this study were tested for blackleg resistance prior to the generation of interspecific hybrids. The blackleg field data was collected between 1997 and 1999 from a blackleg nursery at Horsham, Victoria, Australia (Purwantara *et al.* 1998, Tables 3-1 and 3-2). Five indicators of blackleg disease were measured at maturity. Survival rate was determined as a percentage of surviving plants at maturity compared to those at five weeks after sowing. Survival rate values exceeding 100% occurred in some slower emerging lines due to plants emerging after the 5-week emergence count (Table 3-1). Blackleg disease incidence, disease rating and lesion lengths were measured on 50 arbitrarily chosen sample plants from the blackleg nursery (Table 3-2). Disease incidence was determined as a percentage of plants showing blackleg symptoms (e.g. blackened lesions), and disease rating was measured by examining a cross section of the base of the stem using a 0-6 scoring system. Ratings of 0 were indicative of no disease, while ratings of 6 indicated complete necrosis of the stem (Purwantara *et al.*, 1998). The nurseries were maintained so that high levels of *L. maculans* spores were consistently present to infect growing *Brassicas* by spreading infected stubble over the area, and cultivating continuous rotations of susceptible *Brassicas* (Purwantara *et al.*, 1998).

Only those *Brassica* species containing the B genome were resistant under the selection pressure of blackleg nursery conditions (Table 3-1). Conversely, no *B. oleracea* (CC) varieties survived (Purwantara *et al.* 1998). Westar and Delta are blackleg susceptible varieties of canola quality *B. napus*. Dunkeld is an Australian blackleg resistant canola quality *B. napus* variety, and Quantum is a Western Canadian blackleg resistant canola quality *B. napus* that was developed at the University of Alberta (Stringam *et al.*, 1995). It should be noted that blackleg tolerance in current 'resistant' canola varieties is considered incomplete, allowing the fungus to survive and reproduce saprophytically even on the most resistant lines. This is thought to avoid undue pressure on the pathogen to overcome current resistance (Salisbury, P. *pers. comm.*). The most blackleg resistant *B. napus* cultivar was Dunkeld with an average survival rate of 67%. Other *B. napus* varieties were significantly less resistant, with Quantum showing an average of 10.7 % survival. The relationship between blackleg nursery survival and actual internal fungal growth was established by measuring the internal lesion sizes of the *Brassica* lines grown in the blackleg nursery (Table 3-2). B genome containing species showed significantly less hyphal extension and lesion size than non-B genome containing species

(Purwantara *et al.* 1998). These findings confirmed previous research in demonstrating sources of blackleg resistance exist within the *Brassica* B genome.

To confirm Australian field results, blackleg screening was repeated in 2000 using standardized multiple-isolate cotyledon glasshouse blackleg assays at the University of Alberta (Table 3-3). Cotyledon glasshouse assays can be carried out during winter months, are repeatable, accurate and require less space than whole plant assays (Mayerhofer *et al.,* 1997; Stringam *et. al.,* 1995). Both MBX-2 and Topas, were susceptible to the isolate mix, while DH12075 and Quantum exhibited variable reactions to the isolate mixture.

Table 3-1: Performance of various *Brassica* species in the 1998-1999 Australian blackleg nursery. 'Line' is the name of an inbred seed stock. 'Emergence' was counted following a five-week period, after which seedlings were counted. Maturity refers to the number of plants surviving to set seed. '% Survival' was the number of plants that survived to maturity/the number of plants that emerged in the first 5 weeks following seeding. In cases where more that 100% survival was reported, some plants germinated past the five-week emergence count and survived to maturity (Purwantara *et al.*, 1998).

Line	Species	*Replication*	*Emergence %*	*Maturity*	*% Survival*
Delta	*B. napus*	1	77	1.0	1.3
Delta	*B. napus*	2	66	1.0	1.5
Delta	*B. napus*	3	72	3.0	4.2
Delta	*B. napus*	4	92	3.0	3.3
		mean	77	2.0	2.6
Quantum	*B. napus*	1	49	3.0	6.1
Quantum	*B. napus*	2	44	1.0	2.3
Quantum	*B. napus*	3	58	18	31.0
Quantum	*B. napus*	4	31	1.0	3.2
		mean	46	6.0	11.0
Dunkeld	*B. napus*	1	120	80	67.0
Dunkeld	*B. napus*	2	77	61	79.0
Dunkeld	*B. napus*	3	78	51	65.0
Dunkeld	*B. napus*	4	80	44	55.0
		mean	89	59	67.0
Westar	*B. napus*	1	51	2.0	3.9
Westar	*B. napus*	2	70	1.0	1.4
Westar	*B. napus*	3	64	2.0	3.1
Westar	*B. napus*	4	43	2.0	4.7
		mean	57	1.8	3.3
BCA-065	*B. carinata*	1	63	66	105.0
BCA-065	*B. carinata*	2	52	43	83.0
BCA-065	*B. carinata*	3	53	88	166.0
BCA-065	*B. carinata*	4	83	82	99.0
		mean	63	70	113.0
BCA-070	*B. carinata*	1	51	82	160.0
BCA-070	*B. carinata*	2	50	43	86.0
BCA-070	*B. carinata*	3	82	93	113.0
BCA-070	*B. carinata*	4	59	57	97.0
		mean	61	69	114.0
Vince	*B. nigra*	1	27	28	104.0
Vince	*B. nigra*	2	76	85	112.0
Vince	*B. nigra*	3	85	106	125.0
Vince	*B. nigra*	4	67	76	113.0
		mean	64	74	113.0
Torch	*B. rapa*	1	128	0.0	0.0
Torch	*B. rapa*	2	105	1.0	1.0
Torch	*B. rapa*	3	101	2.0	2.0
Torch	*B. rapa*	4	28	1.0	3.6
		mean	91	1.0	1.6
BJU-011	*B. juncea*	1	57	68	119.3
BJU-011	*B. juncea*	2	106	87	82.1
BJU-011	*B. juncea*	3	96	107	111.5
BJU-011	*B. juncea*	4	96	118	122.9
		mean	89	95	108.9

Table 3-2: Disease lesions and resistance of plants grown in the blackleg nursery trials. '% Survival' is the average number of surviving adult plants/ emerging seedlings in a trail plot containing 100 initial seeds. 'Disease incidence' refers to the number of plants showing stem cankers. 'Disease rating' is the average rating (0-6), of a particular line. A rating of 6 indicated lodging resulting in plant death. A rating of 0 would indicate no sign of infection. The mean lesion sizes refer to the average size of all visible cankers. Lesion sizes in Westar were not measured as all of the plants died before maturity (P. Salisbury *pers. comm.*).

Line	*Species*	*% Survival*	*Disease Incidence*	*Disease rating*	*External lesion size (mm)*	*Internal lesion size (mm)*
Westar	*B. napus*	0.00	100.0	6.00	n/a	n/a
Dunkeld	*B. napus*	16.5	98.68	5.62	21.8	6.42
BCA-070	*B. carinata*	96.5	34.14	1.12	0.52	1.27
BCA-065	*B. carinata*	89.0	11.22	0.29	4.74	5.67

Table 3-3: Canadian glasshouse blackleg screens of *B. carinata* and *B. napus* parents. Profit and Quantum were susceptible and resistant controls respectively. Ratings from 0-4 were used, with 4 indicating extreme necrosis of the cotyledon, usually leading to the death of the seedling. Ratings of 0-1 were considered resistant, and ratings of 2-3 were intermediate. It is possible that lines exhibiting more than one type of rating did so as a result of molecular interaction between the *L. maculans* isolates in the spore suspension. For this reason, lines like DH12075 were considered intermediate.

Line	*Blackleg Rating*					*# Plants scored*	*Category*
	0	*1*	*2*	*3*	*4*		
Profit	0	0	0	0	32	32	Susceptible
Quantum	1	20	6	5	0	32	Resistant
DH12075	0	5	3	4	4	16	Intermediate
MBX-2	0	0	0	0	16	16	Susceptible
Topas	0	0	3	2	11	16	Susceptible
BCA-065	0	11	0	0	0	11	Resistant
BCA-070	0	15	0	0	0	15	Resistant

3.2. Development of Interspecific Hybrids

Ten F1 plantlets were derived from reciprocal crosses followed by ovule rescue tissue culture (Table 3-4). Certain cultivars differed significantly in the number of ovules produced and the ability of those ovules to germinate, or form callus. This phenomenon has been observed in the past (Meng and Lu, 1993).

Approximately 100 crosses were made for each parental combination. The number of F1 ovules obtained when *B. napus* was the maternal parent was approximately double the number of ovules obtained when *B. carinata* was the maternal parent. Additionally, the ability of the ovules to generate a callus or germinate normally was also higher when a *B. napus* plant was the maternal parent (19.1% vs. 6.1%). Despite limited sample sizes, there were differences between lines in their ability to produce F1 hybrids. MBX2 was the most prolific maternal parent, generating 25 of the 39 total viable F1 ovules. Conversely, N-o-1 and BCA-070 were relatively unproductive as maternal donors, while Topas failed to generate any ovules as the female parent. Topas' lack of fertility as the maternal parent was also observed in similar interspecific lineages (Bansal, V. *pers. comm.*) This was unexpected as Topas was chosen for its embryogenicity in microspore culture (Pechan and Keller, 1988); however ovule rescue culture differs from pollen microspore culture in many ways. Contamination during long term tissue culture could have affected the germination ratios and the number of resulting hybrids, as the total number of viable ovules was low from the outset. In total, twelve ovules were discarded for this reason (Table 3-4).

The resulting ten mature plants were given designations beginning with "KCN", and each F1 plant was given a number (KCN-1 to KCN-10).

Previous researchers have recognized that interspecific and intergeneric crosses have low success rates if left to seed. For this reason, researchers have focused on using somatic hybridization and tissue culture to further their investigations, and have had to make-do with small population sizes (Li *et al.* 1995, Barret *et al.* 1997). In addition, viable seed can develop following interspecific and intergeneric crossing through parthenogenesis (Ayotte *et al.* 1988). Parthenogenesis (which would result in maternal clones) is a phenomenon which has also been reported in interspecific and intergeneric crosses between *Brassica* species (Eenink, 1974; Dixelius and Forsberg, 1999).

Table 3-4: F1 Hybrid plants resulting from interspecific crosses between *B. carinata* and *B. napus*. The number of ovules rescued indicated all green ovules extracted following four weeks of development on the plant. The number of plantlets to soil was the number plantlets able to survive transplantation to MetroMix. '% Germination' includes plants that germinated or formed callus, by the number of total ovules rescued. 'C' refers to plantlets that became contaminated during tissue culture, and were discarded.

Female Parent	*Male Parent*	*F1 Ovules rescued*	*Germinations or callus development*	*F1 Plantlets to soil*	*% Germination*	*C*	*Resulting Hybrids*
BCA-070	Topas	61	1	1	1.6%	0	**KCN-1**
MBX-2	BCA-065	62	10	7	16.1%	3	**KCN-2,3,4,6,7,8,9**
BCA-065	Topas	27	5	1	18.5%	2	**KCN-5**
MBX-2	BCA-070	45	15	1	33.3%	3	**KCN-10**
BCA-070	N01-01	32	2	0	6.3%	0	**0**
BCA-070	MBX-2	64	0	0	0%	N/A	**0**
N01-01	BCA-070	10	1	0	10.0%	0	**0**
BCA-065	MBX-2	45	3	0	6.7%	1	**0**
BCA-065	N01-01	56	2	0	3.4%	2	**0**
N01-01	BCA-065	10	1	0	10.0%	1	**0**
	Total	**402**	**39**	**10**		**12**	**10**

Thus, the plants obtained from the sexual crosses between *B. napus* and *B. carinata* could have been the product of parthenogenesis. This has been demonstrated in previous studies. For example, Dixelius and Forsberg (1999) performed intergeneric reciprocal crosses between *B. napus* and *A. thaliana*. From a total of 400 bud pollinations, 31 seeds were derived from the *B. napus* female parent and 10 seeds from the *A. thaliana* female. Of the latter, three seeds successfully germinated, although none of the seedlings survived. By contrast, all 31 reciprocal seeds developed into mature plants. Three of these plants were male-sterile, while the remaining 28 were fertile. Southern hybridization revealed that none of the putative hybrids contained either whole chromosomes or intact chromosome arms of *A. thaliana*. Only one of the plants showing very low fertility hybridized to both *B. napus* and *A. thaliana* molecular markers (Dixelius and Forsberg, 1999). Such results emphasize the need to genetically verify putative hybrids.

Therefore, following tissue harvest of the KCN- putative hybrids, DNA from the ten F1 plants (Table 3-4) were subject to Southern hybridization to verify their hybrid parentage. Four RFLP markers were used to confirm the interspecific crosses, one of which is shown in Figure 3-1. The banding pattern of all F1 plants indicated that they were true hybrids, containing DNA from both parents.

All of the F1 hybrids were vigorous and large, but were sterile, producing stunted or absent anthers and failed to produce self-seed. One hundred self pollinations (bud pollinations) were made on KCN-9 and KCN-10 (200 total) in an attempt to derive F2 seed; however no seed was obtained in either case.

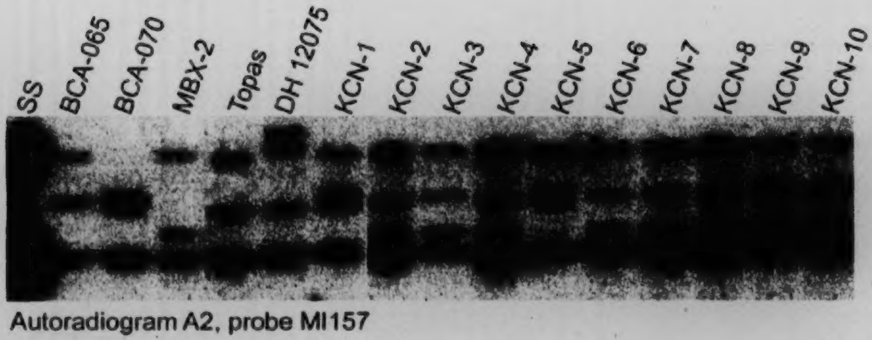


Figure 3-1: Autoradiogram of Eco-R1/RFLP MI157 verifying the interspecific parentage of the 10 F1 plants. SS indicates the size standard, followed by the parents, DH 12075 (*B. napus*), and the ten hybrids. KCN-1 was derived from BCA-070 x Topas, KCN-5 was derived from BCA-065 x Topas, KCN-10 was derived from MBX2 x BCA-070, and the remaining plants were derived from MBX2 x BCA-065.

Due to self-sterility of the F1 plants, backcrosses were necessary to preserve the germplasm. All crosses were made with the F1 hybrid as the female donor and the *B. napus* recurrent parent clone as the male donor, due to the absence of pollen in the F1's. For KCN-1, 3, 5 and 10, additional backcrosses to DH12075 were made in the hope of mapping the *B. napus* A genome in later generations.

Aside from KCN-1, all competent buds on the primary and secondary racemes of the hybrids were used for bud pollinations. The number of these pollinations varied primarily due to the different numbers of buds per raceme present on some hybrids. If the number of competent buds was low on a particular hybrid plant, some crosses were made using clones of the original F1 hybrid. The number of siliques that survived to maturity would have been influenced by many factors, but a proportion of fertilized racemes were lost due to morphological abnormalities present in some of the hybrids. In particular, many of the hybrids and some of the subsequent backcross families exhibited osmotic abnormalities that caused some raceme stems to wilt, while other parts of the same plant would display a type of over-turgidity that caused some stems to spontaneously crack or burst (Figure 3-2).

Siliques that wilted and died from turgid stem wilt were removed from the plant and were not considered in fertility calculations. The remaining silique yield varied from 0.08 of the bud pollinations resulting in a developed silique (KCN-3), to 0.68 (KCN-9), with a mean of 0.38 of the bud pollinations resulting in a developed silique. In normal siliques, the presence of seed within a silique results in a tubular silique, while an empty silique leads to a flat underdeveloped silique. Even the presence of a single developing seed usually results in a localized bulge in the silique. In the case of the hybrid germplasm, the presence of fully developed siliques was not always indicative of the number, or presence of seeds in the siliques. This phenomenon could be observed in hybrids such as KCN-9, which had a large number of tubular developed siliques but little seed. This trait also varied depending on the hybrid: KCN-1 siliques developed normally (looked full) even if there was at least one aborted seed inside the silique. The ability of the hybrid to maintain its fertilized siliques was also variable and is directly compared in Table 3-5, where the average number of buds pollinated on each raceme was related to the number of siliques that matured on that particular raceme.

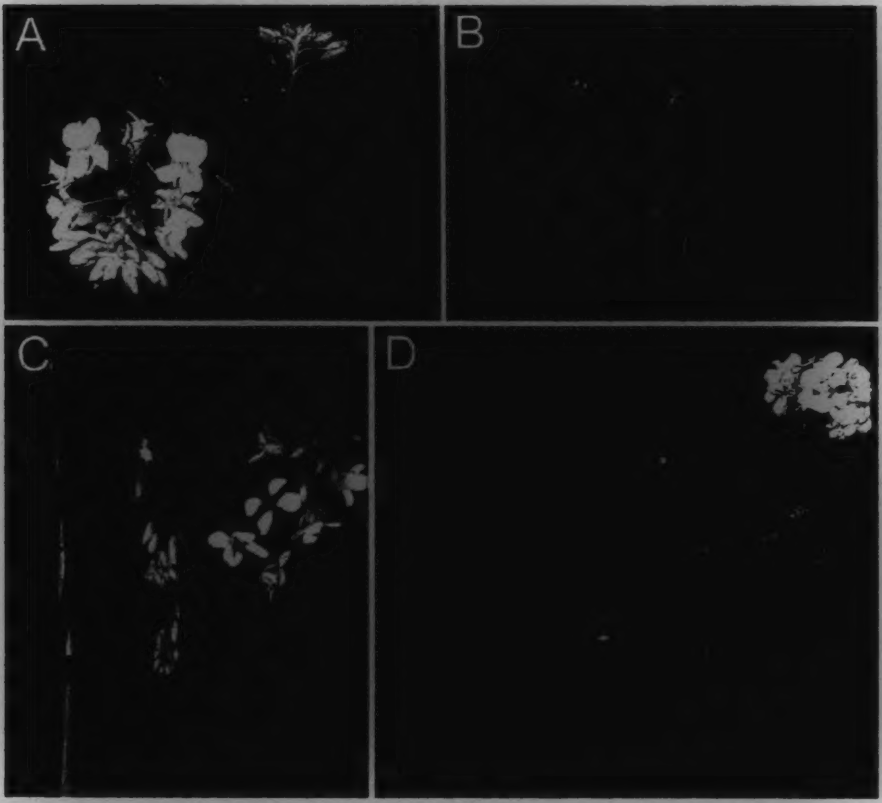


Figure 3-2: Turgid stem wilt in the hybrid plants. A: KCN-10-2-11-52-3 (BC3F2) showing the signs of turgid stem wilt. Note the regional area of wilt and stem 'weeping', while the other stems remained turgid. The simultaneous cracking of the main stem is shown in **B**. This raceme would progressively wilt over 24-72 hours until the localized area on the stem vasculature collapsed and the raceme died as shown in **C**. **D:** KCN-10-2-11-30-9 (BC3F2) showing osmotic disruption of the main stem and other racemes causing abnormal development.

The practical fertility was calculated using the number of seed obtained, over the number of crosses originally placed on the hybrid. It was expected that normal fertility should result in at least 12.5 seeds per bud pollination (cross), and close to 100% germination immediately following harvest (Table 3-6). Sample reciprocal bud pollinations between the *B. napus* varieties 'Shiralee', 'Maluka' (Mayerhofer *et al.*, 2005) and 'MBX-2', resulted in 9 to 27 seeds per bud pollination

(Mayerhofer, M. *pers. comm.*). For the F1 hybrid backcrosses, this fertility was on average 0.06 seeds/ cross. This number was also variable, with the most fertile hybrid (KCN-1 X Topas and KCN-1 X DH12075) producing 0.16 seeds per cross, and the least fertile (KCN-8 X MBX-2) producing 0.02 seeds/cross. Based on the number of BC1 seed available, and the predicted fertility of the next generation, it was decided that the KCN-5 BC1 plants would be used to generate the first linkage map.

Table 3-5: Bud pollinations on F1 hybrid plants. BC1 crosses refer to the number of bud pollinations left to mature to seed on the F1 plants. The number of 'BC1 siliques' indicated the number of siliques that survived on the plant to maturity. The number of seed harvested is the total number of seed taken from the surviving siliques. The percentage of 'siliques/ #crosses on raceme' refers to the number of surviving siliques present at maturity versus the number of bud pollinations made on that raceme. BC1 seed planted is the number of seeds that were placed on damp sand to be germinated, while the actual germinations and resulting adult plants were also recorded. The KCN-5 and KCN-10 backcross statistics are in bold as they are the progenitors of the mapping populations.

Hybrid Female Parent	*BC1 Crosses*	*BC1 Siliques*	*BC1 Seed*	*BC1 Seed/ Cross*	*BC1 Siliques / #crosses on raceme*	*BC1 Seeds planted*	*BC1 Germin-ations*	*Germination (adult plants/seed)*	*#of BC1 plants*	*Plants /Cross*
KCN-1	587	191	88	0.15	n/d	88	32	31%	27	0.04
KCN-2	156	55	15	0.10	87.5%	15	10	40%	6	0.04
KCN-3	218	17	8	0.04	71.3%	8	4	50%	4	0.02
KCN-4	205	58	9	0.04	35.0%	9	6	56%	5	0.02
KCN-5	**356**	**201**	**52**	**0.15**	**63.6%**	**52**	**41**	**77%**	**40**	**0.11**
KCN-6	262	142	16	0.06	66.1%	16	10	50%	8	0.03
KCN-7	141	47	6	0.04	54.0%	6	2	33%	2	0.01
KCN-8	248	90	5	0.02	34.9%	5	2	40%	2	0.01
KCN-9	299	202	8	0.03	89.4%	6	3	50%	3	0.02
KCN-10	**344**	**86**	**11**	**0.03**	**42.4%**	**11**	**8**	**64%**	**7**	**0.02**
KCN-1	118	74	19	0.16	67.3%	19	8	32%	6	0.05
KCN-3	109	n/d	8	0.07	n/d	7	3	43%	3	0.03
KCN-5	106	85	17	0.16	n/d	8	4	50%	4	0.04
KCN-10	125	95	8	0.06	74.3%	16	5	31%	5	0.04
Total crosses	**3274**		**270**					**Total BC1 plants**	**122**	

Table 3-6: Bud pollinations on parental plant lines. Self-bud pollinations were placed on parental plants as a control for bud pollination and germination rates. The number of selfed crosses indicated the number of self-bud pollinations made on a mixture of primary and secondary racemes. The number of seed was denoted as greater than 500 seed. A subset of 40 seed was then germinated on sand, and the percentage of germinated seed was recorded.

Line	*# Self bud pollinations*	*# seed*	*Germination (adult plants/seed)*
Topas	40	>500	100%
MBX-2	40	>500	100%
BCA-070	40	>500	85.7%
BCA-065	40	>500	100%
DH12075	40	>500	100%

3.3. The development of a BC1 mapping population derived from *B. napus* and *B. carinata*

Molecular data derived from a BC1 mapping population would resolve genomic changes that occurred early in the interspecific breeding process, and would have a high probability of segregating for blackleg resistance (Lydiate, D. *pers. comm.*). However, a BC1 population could also have had a higher probability of expressing agronomic *B. carinata* characteristics and transgressive traits. Also, early generation interspecific hybrids are known to be relatively infertile (Rieseberg *et al.*, 2000; Harsh *et al.*, 2007).

Of the original ten lineages, the KCN-5 BC1's and the KCN-1 BC1's were the only sibling families large enough to create linkage maps. KCN-5 BC1's were eventually chosen based on fertility and germination factors. It was not possible to score the F1 hybrids for blackleg resistance as they were derived from tissue culture, and did not develop from normal embryos. F1 plants also failed to produce sufficient selfed seed for indirect blackleg screening. Furthermore, cotyledon screens of the BC1's could have killed the mapping population plants prior to tissue/seed harvest. Hence, some BC1's were scored using subsets of BC2 seedlings (Appendix B). In particular, resistance data was used to select a resistant KCN-5 BC1 plant for further backcrossing; KCN-5-2 (Table 3-7).

Of the 40 BC1 individuals comprising the KCN-5 mapping population, five

BC2 families (representing five of the 40 KCN-5-BC1 plants) were successfully scored for Canadian blackleg resistance using the multiple isolate method (Table 3-7) (Stringam *et al.*, 1995). Following cotyledon blackleg rating, the infected cotyledons from resistant plants were removed to preserve the plant for tissue harvest and further backcrossing. Surviving susceptible BC2 plants were rescued by removing the primary meristems, and cultivating them on new media. Nevertheless, most susceptible seedlings did not survive. All surviving individuals were grown for tissue harvest and additional backcrossing.

The KCN-5 (BC2) sibling population was derived from the BC1 plant KCN-5-2, and was chosen to be included on the KCN-5 mapping population SSR gels. The KCN-5 sibling population (KCN-5-2-1 to KCN-5-2-9) was developed and analyzed with molecular markers in order to detect the presence or absence of characterized alleles in the generation following the KCN-5 mapping population. This sibling SSR assay information was also subsequently used to survey for translocations (none were detected), and the SSR assay matrix was used to corroborate allele assignments in the mapping populations (Appendix A). KCN-5-2 was morphologically well suited to act as a sibling population progenitor. It was also a blackleg resistant plant from which I obtained a significant amount of genomic DNA, an average amount of BC2 seed, and produced no F2 seed. It should be noted that some BC1 plants produced BC and F2 seed but did not produce leaf tissue suitable for DNA isolation.

Most of the KCN-5 BC1 plants in this population were self-sterile, and/or had morphological flowering anomalies that inhibited the production of F2 seed (Table 3-7). Nevertheless, ten BC1 plants did produce F2 seed. BC1 plants either produced self-seed easily, or produced a few seed. The ability of plants to produce BC2 seed also varied greatly; from 0 seeds/ cross to 7.85 seeds/ cross. All of the BC1 plants that were able to produce substantial F2 seed were also able to produce higher numbers of BC2 seed. It was originally assumed that higher fertility would be associated with less *B. carinata* introgression. However, three of five BC1 plants that were scored as blackleg resistant produced significant amounts of F2 seed

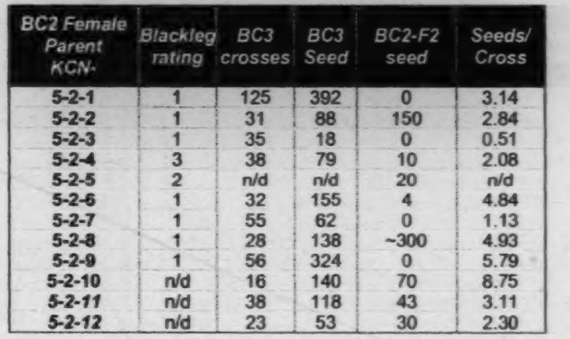
(Table 3-7). Germination data was only collected from families which were scored for blackleg resistance. From this data, germination and seed fertility were correlated to blackleg resistance (Figures 3-3 and 3-4, discussed in section 3.4.).

Fertility was found to increase with each backcross generation (Table 3-8). The average proportion of seeds per cross increased from 0.03 seeds/ cross in the F1 generation (Table 3-5), to 2.3 seeds/cross in the KCN-5 mapping population (Table 3-7), to 3.6 seeds/ cross in the BC2 plants (Table 3-8). I also observed an increase in the BC2 population's ability to produce selfed seed.

Table 3-7: The KCN-5 mapping population: bud pollinations and fertility data. All BC1 plants were backcrossed to a recurrent parent clone (Topas), HC individuals were backcrossed to DH12075. 'C2 crosses' refer to the number of backcrosses placed on each BC1 plant, 'n/d' (no data) indicates that the plant died or produced infertile flowering racemes. 'Crosses harvested' referred to the number of surviving siliques at maturity, and the number of BC2 siliques referred to the actual number of swollen siliques present at maturity that produced BC2 seed. 'n/g' means that none of the seed was germinated ('Germ'.). BC2 progeny from KCN-5-2 were chosen to continue the introgression process.

BC1 Female Parent KCN-	*BC2 Crosses*	*BC2 Crosses harvested*	*BC2 Siliques*	*BC2 Seed*	*BC1-F2 seed*	*BC2 Seeds/ Cross made*	*Plants/ seed used*	*% Germ.*	*blackleg rating*
5-01	61	61	38	102	0	1.67	n/g		
5-02	**57**	**47**	**28**	**43**	**0**	**0.75**	**13/ 43**	**30.2%**	**R**
5-03	n/d	0	0	0	0	n/d	n/g		
5-04	10	n/d	n/d	0	0	0.00	n/g		
5-05	n/d	0	0	0	0	n/d	n/g		
5-06	53	53	46	291	0	5.49	n/g		
5-07	58	52	1	1	0	0.02	n/g		
5-08	56	53	3	9	0	0.16	n/g		
5-09	16	n/d	n/d	0	0	0.00	n/g		
5-10	n/d	0	0	0	0	n/d	n/g		
5-11	n/d	0	0	0	0	n/d	n/g		
5-12	22	n/d	n/d	0	0	0.00	n/g		
5-13	59	58	21	9	0	0.15	n/g		
5-14	51	41	41	263	~200	5.16	58/ 65	89.2%	R
5-15	59	59	34	49	0	0.83	n/g		
5-16	9	9	n/d	45	22	2.44	n/g	n/d	R
5-17	65	65	36	162	0	2.49	n/g		
5-18	74	74	40	61	0	0.82	n/g		
5-19	n/d	0	0	0	0	n/d	n/g		
5-20	53	40	37	162	3	3.06	n/g		
5-21	37	37	19	28	0	0.76	n/g		
5-22	69	63	20	58	5	0.84	n/g		
5-23	n/d	0	0	0	0	n/d	n/g		
5-24	53	n/d	n/d	52	0	0.98	n/g		
5-25	51	n/d	n/d	76	0	1.49	n/g		
5-26	50	50	29	141	35	2.82	n/g		
5-27	55	55	46	143	0	2.60	n/g		
5-28	104	104	48	93	0	0.89	n/g		
5-29	46	46	18	159	2	3.46	n/g		
5-30	42	42	37	486	0	11.6	n/g	n/d	S
5-31	15	11	9	171	0	11.4	n/g	n/d	S
5-32	53	n/d	n/d	53	0	1.00	n/g		
5-33	66	66	35	88	0	1.33	n/g		
5-34	46	46	26	23	0	0.50	n/g		
5-35	8	8	8	6	0	0.75	n/g		
5-36	67	49	43	314	5	4.69	n/g		
5-37	84	84	38	54	0	0.64	n/g		
5-38	73	63	32	314	~100	4.30	n/g		
5-39	51	51	44	291	0	5.71	n/g		
5-40	34	33	7	25	0	0.74	n/g		
5-HC-01	26	26	23	204	~200	7.85	n/g		
5-HC-02	33	33	25	57	0	1.73	10/ 50	20.0%	R
5-HC-03	53	n/d	n/d	196	0	3.70	n/g		
5-HC-04	13	13	11	40	8	3.08	n/g		

Table 3-8: The KCN-5-2 BC2 sibling family: Bud pollinations and fertility data. All BC2 plants were backcrossed to a recurrent parent clone (Topas). 'BC3 crosses' referred to the number of backcrosses placed on each BC2 plant, 'n/d' (no data), indicated that the plant died or produced infertile flowering racemes. Plants were rated directly using the multiple isolate method according to a 0-4 scale; with 0 showing a hypersensitive response, 1 being resistant, 2 and 3 rating as intermediate, and 4 being susceptible.



BC2 Female Parent KCN-	*Blackleg rating*	*BC3 crosses*	*BC3 Seed*	*BC2-F2 seed*	*Seeds/ Cross*
5-2-1	1	125	392	0	3.14
5-2-2	1	31	88	150	2.84
5-2-3	1	35	18	0	0.51
5-2-4	3	38	79	10	2.08
5-2-5	2	n/d	n/d	20	n/d
5-2-6	1	32	155	4	4.84
5-2-7	1	55	62	0	1.13
5-2-8	1	28	138	~300	4.93
5-2-9	1	56	324	0	5.79
5-2-10	n/d	16	140	70	8.75
5-2-11	n/d	38	118	43	3.11
5-2-12	n/d	23	53	30	2.30

3.4. The development of a BC3 mapping population from *B. napus* and *B. carinata*

In theory, a BC3 mapping population should contain lower amounts of introgressed *B. carinata* material and, if selected along with resistance to blackleg resistance, would provide a background containing higher proportions of *B. napus* DNA along with blackleg resistance. To this end, a BC3 mapping population was developed from the hybrid KCN-10. The BC1 plant, KCN-10-2, was chosen based on blackleg resistance data, fertility and morphological observations. This BC1 generated 24 BC2 seeds, and yielded 5 BC2 plants (Table 3-9). One of these BC2 plants (KCN-10-2-11) was chosen, based on a resistant blackleg scoring, to produce a large BC3 population of 200 plants. The BC3 plants were selfed and/ or backcrossed to produce both F2 and BC4 seed (Appendix C). A subset of 150 individuals were rated for resistance to *L. maculans* isolates WA74 and WA51, after which 88 BC3's were chosen to comprise the BC3 mapping population. The BC3 plants chosen were selected based on, a) the production of BC4/ BC3F2 seed, b)

DNA harvests, and c) the number of individuals that would fit in the ABI 377 sequencer wells.

Tables 3-9 and 3-10 detail the seed yields obtained for the BC1 and BC2 predecessors to the KCN-10 BC3 mapping population. For both lineages (KCN-5 and -10), the number of backcross seed produced per bud pollination increased each generation. In the KCN-5 lineage; the F1 hybrid displayed a value of 0.15 seeds/ cross, the average value for the BC1 mapping population was 2.4 BC seeds/ cross, whereas the BC3 lines produced 3.65 seeds/ cross (Table 3-8). The success rate of the BC2 crosses could be skewed, in this case, by two high values (11.6 and 11.4 seeds/ cross), however even with these two values removed, the average number of seeds produced per BC2 mapping population plant was 1.77 seeds/ cross. Likewise, the KCN-10 families followed the same pattern. KCN-10 produced 0.03 BC1 seeds/ cross, followed by 0.35 and 1.07 seeds/ cross for BC2's and BC3's respectively. Other lineages showed similar results (Appendix C). These numbers were lower than the control crosses, but rose in each generation following hybridization.

The ability of the plant to sustain bud pollinations to maturity also increased with each generation. BC1 crosses on KCN-10 yielded 86 mature siliques from 344 pollinations (25%; Table 3-5). BC2 crosses ranged from 0% to 94% silique survival, with an average of 60% silique survival for the KCN-10 BC1 lineage (Table 3-9). These figures increased to nearly 100% when considering backcrosses on BC2 plants (data not shown). The KCN-5 lineage had 56% silique survival for the BC1 crosses on KCN-5, 58% for BC2 crosses and near 100% for BC3 crosses. It should be noted that KCN-5 BC2 crosses varied widely, with the lowest silique survival at 1.7% per plant and the highest at 100%.

A significant factor in the number of F2 and BC seed produced was the premature germination of ovules. For all of the hybrid crosses, I observed that many seeds germinated prematurely inside developing siliques, and this lowered fertility. Pre-germinated seed were not counted in seed harvests, so would not affect germination rates, but would have affected the number of recorded seed 'produced'.

Following the blackleg screening of the mapping population lineages, it was possible to correlate blackleg resistance ratings with factors indicative of genome complements. Germination percentages, backcrosses (BC), and self-seed (F2) sets, were compared to the blackleg ratings of some BC1 and BC2 plants. The results of these comparisons are shown in Figures 3-3 and 3-4.

The blackleg ratings of all scored BC1 plants (from both the KCN-5 and KCN-10 population) were correlated to the amount of BC2/F2 seed produced, and the germination of that BC2/ F2 seed (Figure 3-3). Generally, germination rates of BC1 plants that were resistant (rating 1) to blackleg ranged from 9.8% to 100% and there was no obvious relationship between high blackleg resistance and low germination. There was a relationship between low resistance (rating 4) and high germination. In fact, none of the blackleg susceptible BC1 plants suffered from low seed viability. Similar results were observed for F2 seed production, where no visible relationship between low fertility and high blackleg resistance, but self-sterility was only observed when BC1 plants carried blackleg resistance (Figure 3-3). The ability of resistant BC1 plants to support BC bud pollinations was also observed. Resistant plants ranged from 0.4 to 5.2 BC seed/cross, with no value approaching 'wild-type' fertility for backcrossing (Table 3-7). Susceptible plants also ranged in their ability to produce BC seed, however, three out of the five susceptible plants exhibited normal BC fertility (12.5 BC seed/cross), and no plant was infertile. Plants that presented mid-point blackleg ratings generally showed mid-point fertility.

When the same observations were made for BC2 plants, few generalizations could be made. The BC2 plants were all directly screened for blackleg resistance (Figure 3-4), however many of the '4' rated plants did not survive to produce fertility data. Hence, there are limited data points for this rating. It could be said that resistant plants did not approach normal BC fertility (ranged from 0.3 to 5.8 BC3 seed/cross), but could produce F2 seed easily in at least one case. Germinations were only carried out for the BC seed of KCN-10-2-11 (96.6%), so general seed viability data was not available for the other BC2 plants.

Table 3-9: The KCN-10 BC1 sibling family: bud pollinations and fertility data. All BC1 plants were backcrossed to a recurrent parent as indicated. 'BC1 crosses' referred to the number of backcrosses placed on each BC1 plant, 'n/d' (no data) indicated that the plant died or produced infertile flowering racemes. 'BC1 crosses harvested' were the number of crosses that survived to maturity, while 'BC2 siliques' referred to the number of swollen backcross siliques at harvest. Individuals exhibiting two numbers for data (50/57) showed different yields for clones of the same plant. 'BC2' and 'BC1-F2 seed' referred to seed harvested from swollen siliques. The grey segment of the graph referred to BC2 seed that was germinated and screened for blackleg resistance. 'Plants/ seed germ.' was the number of germinations per seed plated for germination. The blackleg ratings were done using BC2 families, and used the cotyledon multiple isolate method.

BC1 Female Parent KCN-	*Male Parent*	*BC2 Crosses*	*BC2 Crosses harvested*	*BC2 Siliques*	*BC2 Seed*	*BC1-F2 seed*	*BC2 Seeds/ Cross*	*Plants/ seed germ.*	*% Germination*	*Blackleg rating*
10-01	MBX-2	54	54	51	10	0	0.19	n/g	n/d	n/d
10-02	MBX-2	59	59	40	24	0	0.41	15/ 21	71.4%	R
10-03	MBX-2	35	35	0	0	1	0.00	n/g	n/d	n/d
10-03	DH12075	21	21	10	10	n/d	0.48	n/g	n/d	n/d
10-04	MBX-2	50	46	23	2	0	0.04	n/g	n/d	n/d
10-05	MBX-2	56/ 28	56/ 28	48/ n/d	50/ 57	56/ 150	1.27	40/ 86	46.5%	R
10-06	MBX-2	62/ 65	62/ 65	53 / n/d	3/ 2	29	0.04	n/g	n/d	n/d
10-07	MBX-2	0	0	0	0	0	n/d	n/g	n/d	n/d
10-HC-01	DH 12075	n/d	0	0	0	0	n/d	n/g	n/d	n/d
10-HC-02	DH 12075	55/ 54	55/ 54	39/ n/d	38/ 34	18/ 46	0.66	n/g	n/d	n/d
10-HC-05	DH 12075	76	42	33	20	5	0.26	n/g	n/d	n/d
10-HC-06	DH 12075	n/d	0	0	0	0	n/d	n/g	n/d	n/d
10-HC-07	DH 12075	51	48	30	8	0	0.16	n/g	n/d	n/d

Table 3-10: The KCN-10 BC2 sibling family: bud pollinations and fertility data. All BC2 plants were backcrossed to a recurrent parent clone of MBX-2. "Backcrosses made" referred to the number of backcrosses placed on each BC2 plant. Only the lineage that was used for blackleg rating was germinated. Plants were rated directly via the cotyledon multiple isolate method on a 0-4 scale. Plants in the last row died shortly after rating due to blackleg infection.

BC2 Female Parent KCN-	*Blackleg rating*	*BC3 Crosses*	*BC3 Seed*	*BC2-F2 seed*	*BC3 Seed/ Cross*	*Resulting BC3 Plants*	*% Germination*
10-2-4	4 (S)	72	38	0	0.53	n/g	n/g
10-2-8	1 (R)	90	125	0	1.39	n/g	n/g
10-2-11	1 (R)	110	306	5	2.78	200/ 207	96.6%
10-2-13	1 (R)	91	31	2	0.34	n/g	n/g
10-2-14	n/d	55	18	0	0.33	n/g	n/g
10-2-(9,10,12)	4 (S)	0	0	0	n/d	n/d	n/d

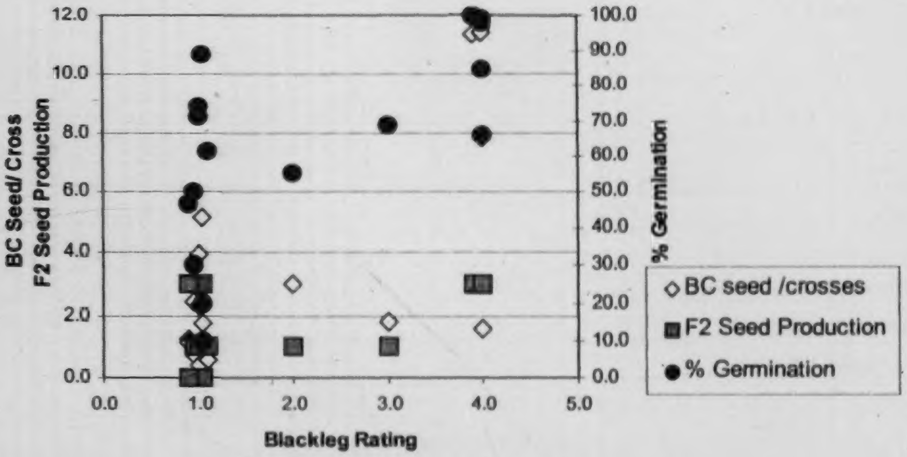


Figure 3-3: The relationship between fertility and blackleg resistance for a group of BC1 plants. Production of BC2 seed, F2 seed and percentage germination, was compared to the blackleg rating of the maternal BC1 plant. Values of 0, 1, 2 and 3 were given to each BC1 plant based on the following; 0=0 F2 seed produced, 1=between 1 and 50 F2 seed produced per plant, 2= between 51 and 100 F2 seed produced and 3=more than 100 F2 seed produced. BC2 seeds/cross was data derived from bud pollinations on BC1 plants. Percent germination was calculated using BC2 seed from each blackleg rated BC1 plant. Where BC2 seed data was absent, F2 germinations were used. For blackleg ratings, BC1 plants were either scored indirectly via BC2 or F2 seed. Blackleg resistance was measured using the multiple isolate method.

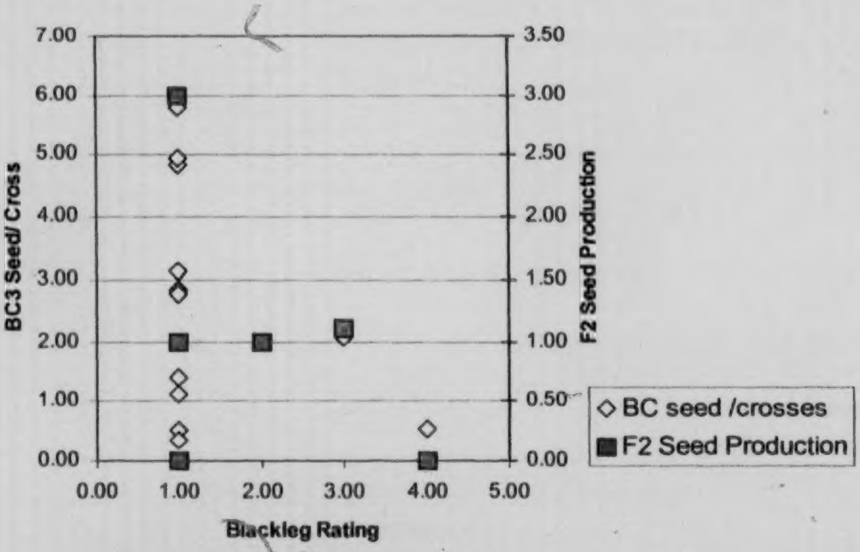


Figure 3-4: The relationships between fertility and blackleg disease resistance for a group of BC2 plants. Production of BC3 and F2 seed was compared to the blackleg rating of the progenitor BC2 plant. Values of 0, 1, 2 and 3 were given to each BC2 plant based on the following; 0=0 F2 seed produced, 1=between 1 and 50 F2 seed produced per plant, 2= between 51 and 100 F2 seed produced, and 3=More than 100 F2 seed produced. BC seed per cross was data derived from bud pollinations on BC2 plants. For blackleg ratings, BC2 plants were scored directly via the multiple isolate method and rescued following screening.

3.6. Summary

By using interspecific bud pollinations and ovule rescue tissue culture, a series of backcross families of interspecific germplasm were produced. F1 hybrids were labeled from KCN-1 to KCN-10. From the ensuing 122 BC1 plants, the lineage derived from KCN-5 was chosen to comprise the BC1 mapping population. All BC1 plants were backcrossed again onto their *B. napus* recurrent parent. A subset of BC1 plants were screened for blackleg resistance and from the results, plants were chosen for further backcrossing. Of the resulting BC2 plants, several of these were chosen for blackleg screening. One resistant BC2 plant (derived from KCN-10), KCN-10-2-11 was then chosen to generate a BC3 mapping population.

KCN-10-2-11 generated a large mapping population (KCN-10 mapping population), while KCN-5 generated a smaller population (KCN-5 mapping population). These lineages were then used for introgression analysis and the generation of further interspecific germplasm. In addition to developing the germplasm, a number of characteristics of the developing lineages were tracked. The fertility of both self and backcrossed pollinations increased each generation, but also varied between individuals. Both mapping population were then used to provide genomic DNA for the molecular analysis of *B. carinata* introgression. The results from the BC1 population introgression analysis, (the KCN-5 mapping population), are presented in Chapter 4, while *B. carinata* introgression in a BC3 population are presented in Chapter 5.

4. Linkage maps and graphical genotypes for the KCN-5 mapping population

The KCN-5 mapping population consisted of a BC1 family of 40 plants derived directly from the hybrid KCN-5 and its recurrent parent; Topas (*B. napus*), as described in Chapter 2. All 40 BC1 plants were germinated, phenotypically evaluated, photographed and had leaf tissue harvested for genomic DNA extraction. These BC1 plants were then allowed to self-seed and were also backcrossed with their Topas parent clone to generate both BC1F2 seed and BC2 seed.

The objective of this section of the project was to track the introgression of the *B. carinata* genome through a set of backcrosses. To achieve this, the first step was to identify microsatellite (SSR) markers that would be informative for each of the interspecific families that had been developed. Specifically, it was important to identify SSR markers (primer-pairs) and alleles (PCR product variant) that would distinguish the C genome from the B genome, as well as individual linkage groups (LGs) within these two genomes. This chapter describes the selection of LG specific markers and the resulting linkage maps and graphical genotypes that were used to ascertain the level of *B. carinata* introgression occurring in BC1 plants.

4.1. Selection and evaluation of SSR markers for the identification of the *Brassica* C and B genomes

The *Brassica napus* C genome has been identified and mapped in a number of *Brassica* populations by several groups and has been determined to comprise nine linkage groups: N11 - N19 (Sun *et al.*, 2007, Piquemal *et al.*, 2005; Parkin *et al.* 1995; Sharpe *et al.* 1995; Bohoun *et al.* 1998; Saal *et al.* 2001; Slocum *et al.* 1990; Landry *et al.* 1991, 1992; Kianian and Quiros 1992; Ferreira *et al.* 1994; and Uzonova *et al.* 1995). More recently, various B genome *Brassica* maps have been developed. The B genome LGs have been defined as comprising eight LGs (J11-J18 or G11-G18) (Axelsson *et al.* 2000; Lagercrantz and Lydiate 1995). These maps were developed using RFLP molecular markers in *B. nigra* or *B. juncea* populations. Since then, the research group at AAFC Saskatoon (Agriculture and Agri-Food Canada Saskatoon), have used *B. napus* and *B. juncea* mapping populations to develop a pool of characterized microsatellite markers which were used in this study (available under material transfer agreement, Lydiate, D. and Sharpe, A AAFC Saskatoon). SSR markers specific to the *Brassica* A genome were present on the AAFC reference maps, however, the A genome could not be mapped as the experimental germplasm was developed through backcrossing to recurrent *B. napus* germplasm (monomorphic for A genome alleles). Primer-pairs that amplified both A and C genome loci (alleles mapped to a known location in the genome) were screened for polymorphisms and included in experimental data.

During mapping work, the assignment of loci to previously established linkage groups is typically made by means of integrating the new linkage map with an accepted map(s) through alignment of common markers (Piquemal *et al.,* 2005; Sun *et al.*, 2007). In this case, there was an absence of interspecific or *B. carinata* mapping populations that could be used for comparison. Even if such template maps existed, alleles in one map derived from certain germplasm can represent different loci in distinct germplasm, even from within the same species. Therefore, markers chosen for this project had to be selected and aligned based on stringent criteria. This included the presence of polymorphic alleles between parents, allele linkage in other published maps of *B. napus* (AACC) or *B. juncea* (AABB), marker amplification behavior during PCR, and whether the allele size, once amplified, corresponded to alleles characterized in the AAFC reference maps.

The procedure for selecting and evaluating SSR markers was as follows. Initially, using the AAFC reference maps (provided by AAFC, Saskatoon), I determined which of the SSR markers would have a high probability of amplifying only one or two loci in the KCN-5 and KCN-10 germplasm. Then markers were selected that mapped at regular intervals along each B and C genome chromosome, including the chromosome tips, according to the AAFC reference maps. Once these markers were selected, the primers for each SSR marker were evaluated using a set of Screening Gels (SG) which compared the band sizes of the amplified alleles in experimental populations to those characterized in the AAFC reference populations and representative *B. nigra* (BB), *B. oleracea* (CC) and *B. rapa* (AA) DNA samples.

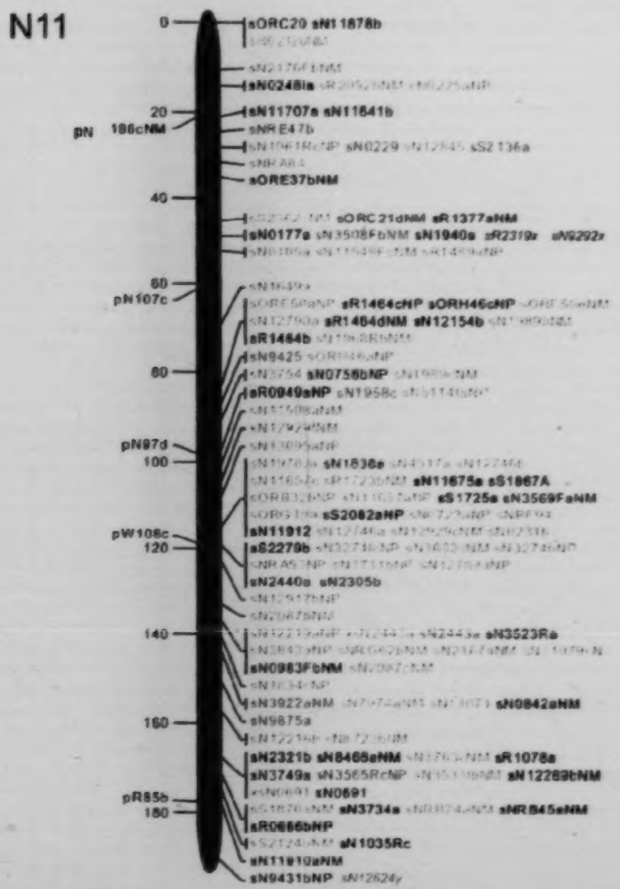
Representative diploid *Brassica* samples were also included on the SGs: *B. oleracea* ssp. Alboglabra cv. A12 (O'Neill and Bancroft 2000), *B. rapa* ssp. Oleifera cv. R018 (Xiao *et al.*, 2004), and a wild Italian *B. nigra* accession (Lagercrantz and Lydiate 1995). This allowed me to assign alleles to previously mapped loci, and evaluate any primer-pair amplification characteristics that may have affected data interpretation. The SGs were also used to determine whether a marker was polymorphic between the *B. carinata* and *B. napus* parents. Following chromosome alignment in the experimental germplasm, additional characterized AAFC SSR markers were amplified to saturate all of the LGs.

4.1.1. Selecting SSR markers specific to each B and C linkage group

Markers characterized at AAFC were known to amplify loci in several *Brassica* populations including the 61-9 and the 72-8 *B. napus* populations described by Parkin and Lydiate (1997). Mapped loci on these linkage groups provided the preliminary pool of markers, and the framework for the selection of C genome specific markers. Markers amplifying loci on the *B. juncea* T-1 map likewise provided the initial pool of B genome specific markers (Axelsson *et al.* 2000).

Markers were chosen from the collection of loci on these established maps that would span the length of the linkage groups (LGs), and would be distributed along the chromosome at least every 10-20 cM, (5-10 cM in the case of N11, N13, J11, J13), according to the AAFC reference maps available at the time. Figures 4-1 and 4-2 are sample reference LG maps showing the N11 C genome and the J11 B genome linkage groups amalgamated from the 61-9, 72-8 and T-1 linkage maps (D.J. Lydiate and A.G. Sharpe; AAFC Saskatoon Research Centre, Saskatoon SK, Canada; *unpublished*). Markers shown in color were mapped (KCN-5 or -10 mapping population, or both) to N11 or J11, and represent the type of marker distribution achieved for all LGs (Figures 4-1 and 4-2). Terminal LG coverage was especially important to accomplish for each LG, as recombination patterns at the chromosome termini could differ from patterns in the internal part of the chromosome (Schelfhout *et al.* 2006). It should be noted that Figures 4-1 and 4-2 show the markers that were available as of 2006. During this research, almost all available markers on many of the LGs were screened for possible use.

Screening gels, in combination with the AAFC reference maps, permitted the selection of markers based on the number of alleles each marker amplified in a particular genome (Figure 4-3).



N11
0
20
40
60
80
100
120
140
160
180
pN 186cNM
pN107c
pN97d
pW108c
pR85b
sORC20 sN11878b
sN0248Ia
sN11707a sN11641b
sNRE47b
sN0229
sS2136a
sORE37bNM
sORC21dNM sR1377aNM
sN0177a sN1940a sR2319a sN9292a
sR1464cNP sORH46cNP
sR1464dNM sN12154b
sR1464b
sN9425
sN0756bNP
sR0949aNP sN1958c
sN1836a
sN11675a sS1867A
sS1725a sN3569FaNM
sS2082aNP
sN11912
sS2279b
sN2440a sN2305b
sN3523Ra
sN0983FbNM
sN3922aNM sN0842aNM
sN9875a
sN2321b sN8468aNM sR1076a
sN3749a sN3565RcNP sN12289bNM
sN0691
sN3734a sNRB45aNM
sR0666bNP
sN1035Rc
sN11910aNM
sN9431bNP sN12624y

Figure 4-1: N11 reference map of the 61-9/72-8 populations incorporating marker information from KCN-5 and KCN-10 Mapping Populations. Markers in black were screened but were not polymorphic. Markers in grey were not screened. Loci in blue were mapped in the KCN-5 population, orange loci mapped to the KCN-10 population, and pink loci mapped in both experimental populations. Italicized loci were not derived from the AAFC reference LG N11, but were amplified by markers mapping to other AAFC reference LGs. Markers to the left of the LG are reference RFLP markers and the numbers are in m.u. (Lydiate, D. and Sharpe, A. *unpublished*).

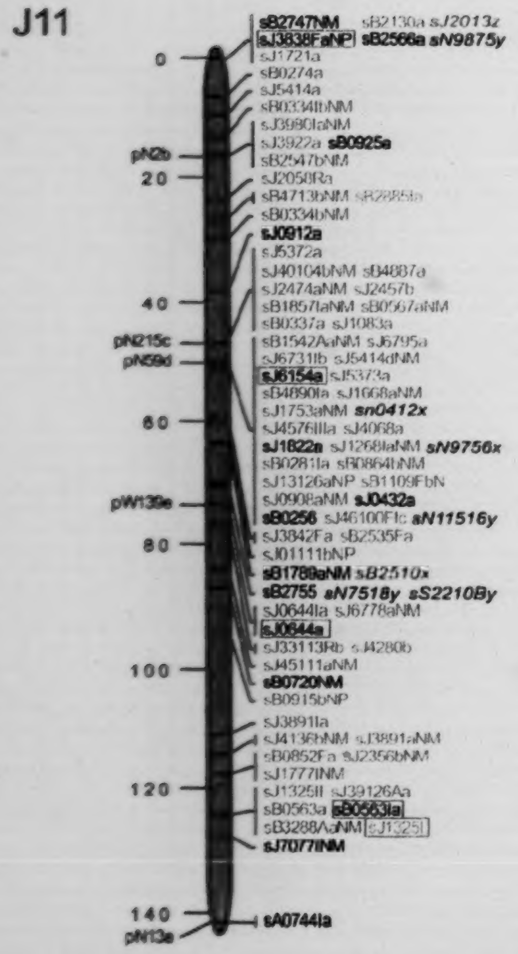

J11
0
pN2b
20
40
pN215c
pN59d
60
pW139e
80
100
120
140
pN13e
sB2747NM sB2130a sJ2013z
sJ3838FaNP sB2566a sN9875y
sJ1721a
sB0274a
sJ5414a
sB0334IbNM
sJ3980IaNM
sJ3922a sB0925a
sB2547bNM
sJ2058Ra
sB4713bNM sB2885Ia
sB0334bNM
sJ0912a
sJ5372a
sJ40104bNM sB4887a
sJ2474aNM sJ2457b
sB1857IaNM sB0567aNM
sB0337a sJ1083a
sB1542AaNM sJ6795a
sJ6731Ib sJ5414dNM
sJ6154a sJ5373a
sB4890Ia sJ1668aNM
sJ1753aNM sn0412x
sJ4576IIIa sJ4068a
sJ1822a sJ1268IaNM sN9756x
sB0281Ia sB0864bNM
sJ13126aNP sB1109FbN
sJ0908aNM sJ0432a
sB0256 sJ46100FIc aN11516y
sJ3842Fa sB2535Fa
sJ01111bNP
sB1789aNM sB2510x
sB2755 sN7518y sS2210By
sJ0644Ia sJ6778aNM
sJ0644a
sJ33113Rb sJ4280b
sJ45111aNM
sB0720NM
sB0915bNP
sJ3891Ia
sJ4136bNM sJ3891aNM
sB0852Fa sJ2356bNM
sJ1777INM
sJ1325II sJ39126Aa
sB0563a sB0563Ia
sB3288AaNM sJ1325I
sJ7077INM
sA0744Ia

Figure 4-2: J11 reference map of the T1 population incorporating marker information from KCN-5 and KCN-10 Mapping Populations. Markers in black were screened but were not polymorphic. Markers in grey were not screened. Markers in blue were mapped in the KCN-5 population, orange loci mapped to the KCN-10 population, and pink loci mapped in both experimental populations. Italicized loci were not derived from the AAFC reference LG J11, but were amplified by markers mapping to other AAFC reference LGs. Markers to the left of the LG are reference RFLP markers and the numbers are in m.u. (Lydiate, D. and Sharpe, A. *unpublished*).

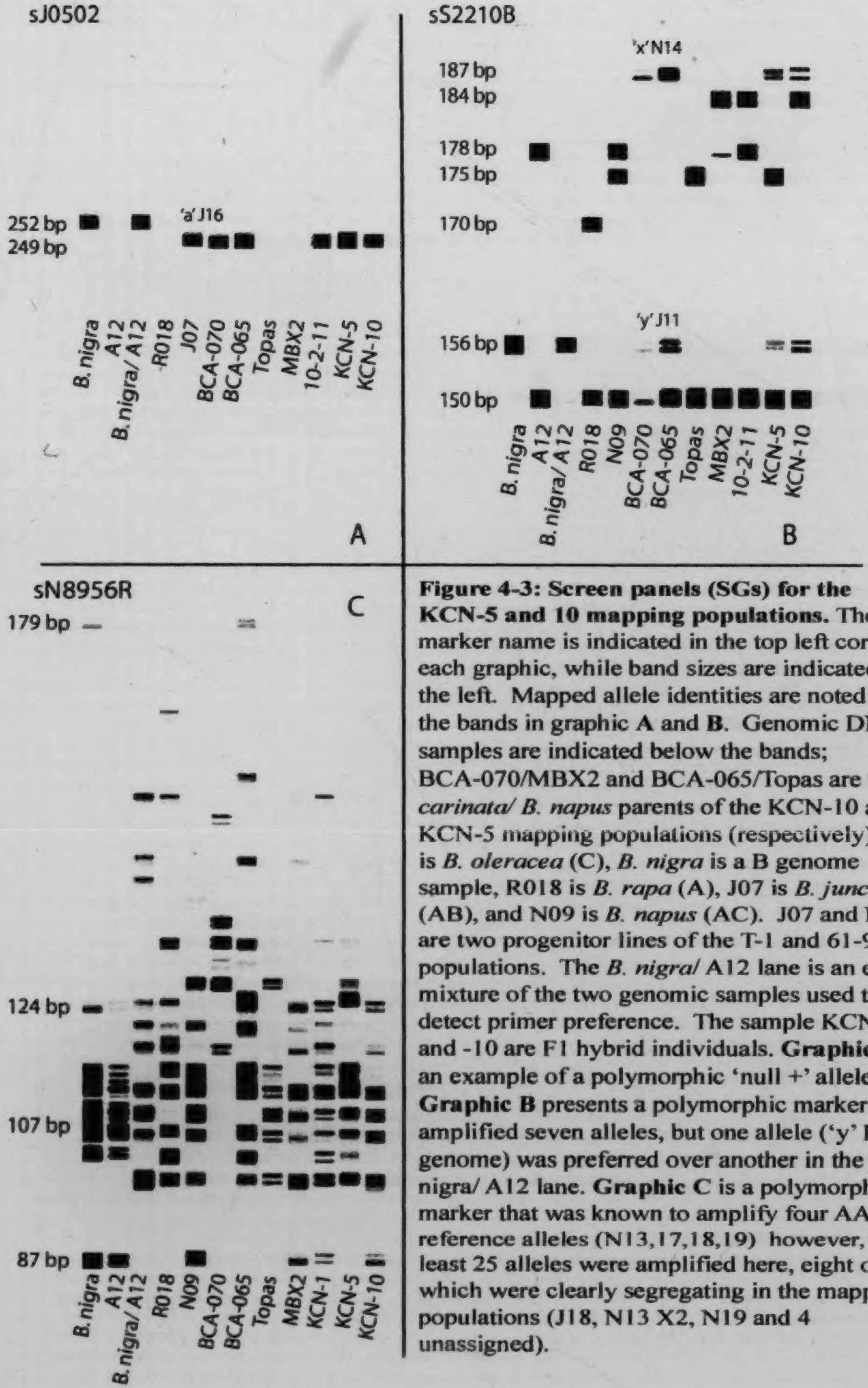

sJ0502
'a'J16
252 bp
249 bp
B. nigra
A12
B. nigra/ A12
R018
J07
BCA-070
BCA-065
Topas
MBX2
10-2-11
KCN-5
KCN-10
A
sS2210B
'x'N14
187 bp
184 bp
178 bp
175 bp
170 bp
'y'J11
156 bp
150 bp
B. nigra
A12
B. nigra/ A12
R018
N09
BCA-070
BCA-065
Topas
MBX2
10-2-11
KCN-5
KCN-10
B
sN8956R
C
179 bp
124 bp
107 bp
87 bp
B. nigra
A12
B. nigra/ A12
R018
N09
BCA-070
BCA-065
Topas
MBX2
KCN-1
KCN-5
KCN-10

Figure 4-3: Screen panels (SGs) for the KCN-5 and 10 mapping populations. The marker name is indicated in the top left corner of each graphic, while band sizes are indicated to the left. Mapped allele identities are noted above the bands in graphic **A** and **B**. Genomic DNA samples are indicated below the bands; BCA-070/MBX2 and BCA-065/Topas are the *B. carinata/ B. napus* parents of the KCN-10 and KCN-5 mapping populations (respectively). A12 is *B. oleracea* (C), *B. nigra* is a B genome sample, R018 is *B. rapa* (A), J07 is *B. juncea* (AB), and N09 is *B. napus* (AC). J07 and N09 are two progenitor lines of the T-1 and 61-9 populations. The *B. nigra/* A12 lane is an equal mixture of the two genomic samples used to detect primer preference. The sample KCN-1,-5 and -10 are F1 hybrid individuals. **Graphic A** is an example of a polymorphic 'null +' allele. **Graphic B** presents a polymorphic marker that amplified seven alleles, but one allele ('y' B genome) was preferred over another in the B. nigra/ A12 lane. **Graphic C** is a polymorphic marker that was known to amplify four AAFC reference alleles (N13,17,18,19) however, at least 25 alleles were amplified here, eight of which were clearly segregating in the mapping populations (J18, N13 X2, N19 and 4 unassigned).

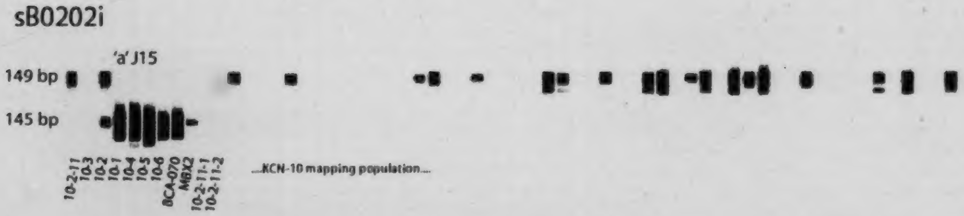


Figure 4-4: Allele shifting in the KCN-10 Mapping Population. The SSR repeat amplified by the marker sB0202i can be seen to extend in KCN-10-2 and its decedents. The allele would also be scored as a 'null+' as it failed to substantially amplify a band in MBX2. The marker name is indicated in the top left corner of the figure while allele size is indicated to the left. The allele identity is noted above the corresponding bands. Genomic DNA samples are indicated below the bands: BCA-070/ MBX2 are the *B. carinata/ B. napus* parents of the KCN-10 mapping population (respectively). 10-1 to 10-6 are KCN-10-BC1 sibling population, 10-2 and 10-2-11(BC2) are the direct progenitors of the KCN-10 mapping population. 10-2-11-1 and onward, are the individuals comprising the KCN-10 mapping population.

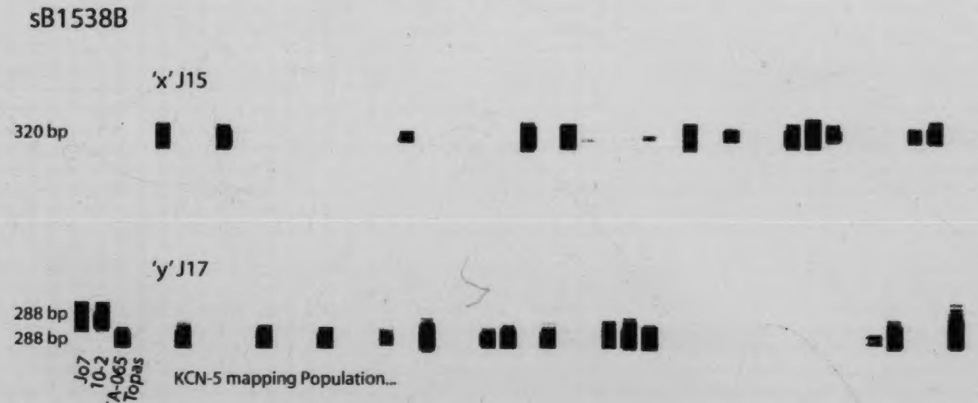


Figure 4-5: Template preference in the KCN-5 Mapping Population. The 'y' (J17) allele amplified by the marker sB1538B was preferred over the 'x' (J15) allele.The allele would also be scored as a 'null plus' as it failed to substantially amplify a band in Topas. The marker name is indicated in the top left corner of the figure while allele size is indicated to the left. The allele identity is noted above the corresponding bands. Genomic DNA samples are indicated below the bands: BCA-065/ Topas are the *B. carinata/ B. napus* parents of the KCN-5 mapping population (respectively). 10-2 is a member of the KCN-10 family, and J07 is one of the parents of the T-1 reference map. The remaining individuals comprise the KCN-5 mapping population. Here, 'y' is scored as a typical 'null plus' allele where the absence of a band can indicate ether a missing *B. carinata* template or a failed PCR reaction. 'x' is only amplified if 'y' is absent hence the absence of the 'x' band does not always mean the absence of that priming template.

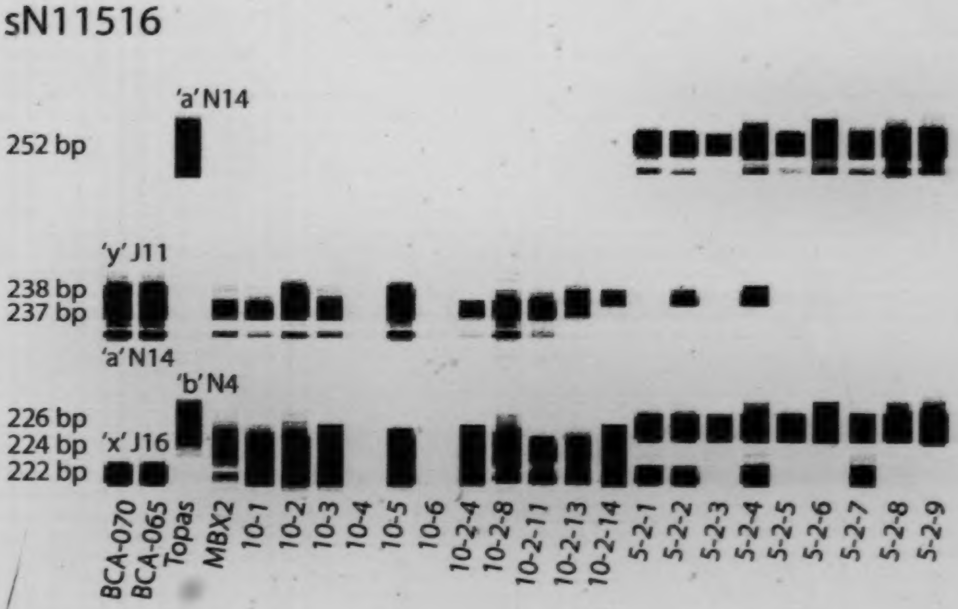

sN11516
'a' N14
252 bp
'y' J11
238 bp
237 bp
'a' N14
'b' N4
226 bp
224 bp
'x' J16
222 bp
BCA-070
BCA-065
Topas
MBX2
10-1
10-2
10-3
10-4
10-5
10-6
10-2-4
10-2-8
10-2-11
10-2-13
10-2-14
5-2-1
5-2-2
5-2-3
5-2-4
5-2-5
5-2-6
5-2-7
5-2-8
5-2-9

Figure 4-6: KCN-5 and KCN-10 mapping population Sibling assay panel. These panels were used to track the presence of *B. carinata* alleles in the three Sibling populations (KCN-5 BC2s and KCN-10 BC1s and BC2s). Characterized markers from these panels were used to assemble the Sibling reference maps. The marker name is indicated in the top left corner of the figure while allele size is indicated to the left. Allele identities are noted above the bands. Genomic DNA samples are indicated below the bands: BCA-070/ MBX2 and BCA-065/ Topas are the *B. carinata/ B. napus* parents of the KCN-10 and KCN-5 mapping populations (respectively). 10-1 to 10-6 are the KCN-10-BC1 Sibling population, 10-2-4 to 10-2-14 are the KCN-10-BC2 Sibling population. 10-2 and 10-2-11 are the direct progenitors of the KCN-10 mapping population. Likewise, 5-2-1 to 5-2-9 are the KCN-5 Sibling population (BC2 descendents of KCN-5-2). Allele 'y' amplified a band in 10-2 but not 10-2-11, and so could not be mapped in the KCN-10 mapping population; however it was mapped in the KCN-5 population family to J11. Allele 'x' was present/segregating in all of the families on the panel and was mapped to J16 in both mapping populations. Allele 'a' was mapped to N14 in the KCN-5 mapping population but was not present in 5-2 and so did not segregate in the BC2 family. Allele 'b' was designated via AAFC reference maps but was not monitored as it amplified a band in the A genome.

During the initial map construction, markers that amplified only one or two known alleles in the AAFC reference maps were chosen until all LGs could be identified and aligned. The occurrence of complex amplification patterns made by most of the markers (Figure 4-3, C) demanded that simple amplification patterns were initially evaluated. Figure 4-3 illustrates a number of the challenges one encounters while evaluating marker amplification patterns. These are discussed in more detail in Section 4.1.3.

The number of markers amplifying one or two bands was small, but they yielded definitive data. For example, of the 52 markers formerly amplifying only one locus on N11 in the 61-9/72-8 populations, 10 amplified a single allele in the experimental germplasm, and 16 amplified only 2 alleles. Although the same allele, or same number of alleles, may not have been amplified in the mapping populations, using reference maps as a resource in this manner simplified the identification of specific linkage groups and informative markers.

4.1.2. Genome identification based on SSR allele amplification size

Following the initial selection of polymorphic SSR markers, SGs were designed to resolve each marker's amplification behavior during PCR, using DNA templates from the different *Brassica* genomes. Due to the possibility of recombination between genomes in the interspecific germplasm, it was important to clarify the identity of the genome being amplified as well as its parental source. For example; in Figure 4-3 (B), the *B. carinata* samples had two polymorphic alleles. The smaller allele 'y' was the same size as an allele amplified from *B. nigra* (BB) and was not amplified by any sample containing only the A or C genomes. Therefore there was a high probability that allele 'y' was from the B genome, and not the C genome in *B. carinata*. As shown, SGs included genomic DNA from all of the progenitors of the KCN-5 and 10 mapping populations as well as genomic DNA controls from species containing only the A (*B. rapa, R018*), B (*B. nigra*), or

C (*B. oleracea, A12*) genomes. A lane was also included that contained a balanced mixture of genomic DNA from the *B. oleracea* and *B. nigra* samples. The latter was included to identify primer preference for one allele over others during PCR (Figure 4-3, B). This will be discussed in more detail in section 4.1.5.

4.1.3. SSR alleles that were amplified in the experimental germplasm and were characterized in the AAFC reference maps

Another pertinent aspect of including various genomes on the SGs was that it permitted the comparison of allele sizes between previously mapped alleles (D.J. Lydiate and A.G. Sharpe, *unpublished*), and the alleles that were amplified in the KCN-5 and 10 mapping populations. The genomic DNA samples of the various reference species (*B. rapa, B. nigra, B. juncea* and *B. napus*) were progenitor lines of other characterized mapping populations (Axelsson *et al.*, 2000; Parkin and Lydiate 1997). The *B. nigra*, *B. oleracea* (A12), and *B. rapa* (R018) lines were characterized by the AAFC Saskatoon Research Centre and were present on other SGs of in-house mapping populations (D.J. Lydiate and A.G. Sharpe, *unpublished)*. The SGs also included genomic samples from characterized *B. napus* (N09) (Parkin and Lydiate 1997), and *B. juncea* (Jo7) lines (Axelsson *et al.* 2000). Alleles that amplified in the KCN mapping populations could be assigned to specific linkage groups based on the PCR product size (supplementing traditional LG assembly). This was possible if the band size matched the size of previously mapped alleles of the AAFC reference maps. Alleles assigned to specific chromosomes could then be used to assay F1 hybrids and BC1 and BC2 sibling families for the presence or absence of specific linkage groups or linkage group fragments. Figure 4-6 is an example of a sibling panel that was used to construct graphical genotypes of the sibling families (Section 4.4.).

The KCN-5 mapping population scoring data generated 304 loci, of which 113 were categorized as previously mapped alleles in the AAFC reference maps. These were initially labeled by the appropriate AAFC reference map suffix 'a', 'b', or 'c'. It was later determined whether the 113 AAFC alleles had been assigned to the appropriate location after the KCN-5 linkage map was saturated. Only three were eventually found to map to alternate loci. Thus, there was a high probability of correctly assigning an allele based solely on matching the experimental allele size to the AAFC reference map allele size (*prob.* =0.973). Alleles that had not been previously mapped in the AAFC reference populations were given a suffix ranging from 's' to 'z'. Altogether, 406 primer pairs were initially screened with the experimental germplasm using SGs. Of these, 270 markers were selected for further analysis from the pool of A/C genome specific markers derived from *B. napus*, (with an average of 30 markers per linkage group), and 136 markers were selected from the pool of A/B genome specific markers (with an average of 17 markers per linkage group) derived from *B. juncea* and *B. nigra.*

4.1.4. SSR primer template preference

Of a subset of 220 arbitrarily chosen markers (SSR subset), (amplifying 344 alleles in the experimental germplasm), it was determined that 69 of the primer-pairs (31%) exhibited a priming preference for a particular allele over another. SSR primer-pairs derived from particular germplasm did not guarantee that the same markers would prefer to amplify like-germplasm when presented with alternate templates. For example, within the SSR subset, 9 alleles (3%) were derived from markers developed from *B. napus* (AACC) germplasm, but preferred *B. carinata* C genome loci within the interspecific germplasm. In our subset, there were no examples of B genome specific primers that preferred C genome templates.

Also, markers amplifying loci in their progenitor genome, as well as in other genomes, was highly probable. In the 220 SSR marker subset, 43 of the mapped alleles were amplified in the B genome of *B. carinata,* but were originally derived from *B. napus* (AACC) germplasm. Likewise, 10 alleles were amplified in the *B. carinata* C genome that originally came from *B. juncea* (AABB) germplasm. In total, 15.4% of the mapped alleles were amplified by primer pairs that were developed from germplasm that did not include the specific genome which they amplified in the experimental germplasm. For example, Figure 4-3 (C) illustrates a marker that was developed from *B. napus* genomic DNA, but amplified multiple bands in all genomes.

4.1.5. PCR-based artifacts and null plus alleles

Several other PCR-based artifacts were encountered which had to be assessed to avoid misinterpretation of the data and to evaluate the behavior of certain primer pairs. To begin, certain loci occasionally failed to amplify under certain PCR conditions related to the thermocycler used. Even when the same PCR parameters were applied, several primers showed inconsistent amplification. This became significant, for example, if a marker was expected to generate a band representing a known locus on J13, but five lanes near the plate edge failed to amplify that band due solely to a change in the thermocycler-PCR plate interface. If this were to occur, the recombination frequency between this locus and its adjacent locus could become artificially inflated by 12.5 cM, since antomated LG assembly software would assume that I was evaluating 40 recombinant lines, not the 35 true data points, thus inflating the recombination values. Along the same line, larger PCR products (~500bp) occasionally failed to amplify if the sample was adjacent to the plate edge. This inconsistent amplification could be detected through observing SGs and Sibling screening panel banding patterns, (different thermocylers and well positions during PCR), and increasing the visual intensity of irregular alleles.

While inconsistent amplification was rare, a common occurrence was a *B. juncea* derived marker failing to amplify any loci in the *B. napus* parent. Scoring for these markers consisted of assigning plus(+) for the appearance of the allele, and a 'null'(v) for the lack of the allele. The null assignment (v) corresponded to either an absent allele ('-'), or a failed PCR amplification ('0'). SSR primer pairs that only amplified alleles in the *B. carinata* germplasm were referred to as 'null plus' (NP) alleles, and were given a 'NP' suffix (i.e. sB1828NP). In the scoring matrices, NP alleles were converted to minus (-) assignments if the loci above and below the NP allele were also minus assignments (hence a plus assignment would be a double-crossover, and therefore unlikely). If the alleles above and below the NP allele were different (+,-) or both plus (+,+), the NP allele was changed to a zero (0). These allele assignment changes were made because, to the best of my knowledge, there are no mapping programs that can accommodate undefined data (NP). Figure 4-3 (A) is an example of a NP allele on a screen gel. Furthermore, NP alleles could occur in concert with other factors, such as template preference (Figure 4-5).

Natural genetic phenomena could also influence correct scoring. For example, Figure 4-4 shows a marker that amplified a lengthening repeat generated during the meiosis of KCN-10-2. This longer repeat was then inherited in subsequent generations.

Hence, the SGs were used to find informative markers via polymorphic alleles, provide initial information about the genomic identity of each allele, and predict primer-pair reactions to the presence of different genomic templates.

Through the use of established maps, analysis of allele size, and SGs that incorporated genomic material from various *Brassica* species, it was possible to derive more information from each marker than would be yielded via random screening for polymorphic amplification. It was also possible to ensure relatively even coverage throughout the LGs of the KCN-5/ 10 mapping populations, resulting in a map that would have otherwise required many more markers to achieve the same saturation.

4.2. Assembly of linkage groups comprising the C and B Genomes of the KCN-5 Mapping Population

The C and B genome linkage groups of the KCN-5 mapping population were designated by aligning putative linkage groups with established SSR maps of C and B genome components via common marker alleles derived from the AAFC microsatellite markers. Occasionally there was insufficient power in the C genome data to link C genome segments together. The identities of the segments were, however, well established on the basis of shared marker and allele identities. Thus, the segments are presented in their correct alignments and locations. In these cases, the recombination events between segments were manually determined (Figures 4-7 to 4-8). For the C genome, 26 spans were manually determined. In 54% of cases, the RF for these spans indicated intermediate linkage (20-35 cM), in 42% of cases, loose linkage (36-45 cM), and in one case, independent assortment (>45 cM).

The KCN-5 population linkage map of the B and C genomes consisted of 17 linkage groups, 290 alleles and spanned 1959.0 cM. A subset of 110 shared AAFC reference map/KCN-5 loci were used to align and orient the KCN-5 mapping population linkage map. The putative linkage groups were also anchored with a collection of SSR markers that are publicly available, (AAFC Saskatoon), these in turn have been aligned with RFLP linkage maps (Parkin *et al.* 1995 and Axelsson *et al.* 2000). The C genome linkage groups were defined as N11 to N19 (Figure 4-7 and 4-8), while the B genome linkage groups were labeled J11 to J18 (Figure 4-9). The data also contained 14 independent unassigned marker alleles. Approximately eight of these unassigned loci were excluded solely due to ambiguous PCR amplification patterns. Figure 4-9 shows the B genome LGs as they would appear if only RF values were used to align and space mapped loci. Since the B genome chromosomes were not undergoing recombination, as traditionally defined, it was not possible to align loci based solely on data obtained from the scoring matrix (Appendix A). For simplicity sake, RF values derived from the B genome scoring matrix were referred to as pseudo-recombination (*p*RF). *p*RF was the actual loss of terminal loci interpreted as recombination by mapping software functions. The LGs are presented in Figure 4-9 as clustered/ co-mapping loci to illustrate how B genome chromosomes could appear as an introgressed segments of clustered loci if *p*RF values are used to assemble LGs. This issue will be discussed further in Chapter 6.

The B genome linkage groups ranged from *p*RF =11.7 to 19.5 cM. The largest non-coincident span between two markers was 12.4 cM (less than five pseudo-recombination events between markers), with the majority of non-coincident spans (77%), consisting of only one pseudo-recombination event between two markers.

All of the mapped loci were present in the F1 hybrid KCN-5. Alleles sR6083b (N16, Figure 4-8) and sJ46102y (J16, Figure 4-9) amplified in the F1, but failed to amplify in successive generations. Both of these loci were located terminally in the reference maps. Thus, it was possible that these loci were lost in F1 meioses, or the variation in the PCR conditions between SGs (ABI 877) and the thermocyclers used to amplify the mapping PCR samples caused the failed amplifications.

4.3. Segregation distortion in the KCN-5 mapping population

The scoring matrix (Appendix A) was used to detect preferential inheritance of particular loci or linkage groups. Marker scorings for each locus were tested for segregation distortion using a chi-squared (x^2) statistical test. Table 4-1 and 4-2 shows the $x^2_{1:1}$ results for loci in both the B and C genome linkage groups, respectively. The majority of loci mapping to the C genome did not display significant segregation distortion. Of the six C genome loci that displayed segregation distortion, a small region on N13 (sN8956Rw, sORC95b) displayed an increased inheritance of *B. carinata* alleles, while a small region on N14 (sORH43a, sN3508Fw) showed an increased inheritance of *B. napus* alleles. The other two loci displaying segregation distortion were independent. Most C genome segregation distortion could be attributed to the location of missing data points (i.e. N18).

Allele segregation on some B genome linkage groups was distorted ($p < 0.05$). J12, J15 and J16 displayed significant distortion through the entire chromosome, indicating that the chromosome was preferentially inherited more frequently than would be expected ($\chi^2_{1:1}$). A few additional alleles did exhibit segregation distortion which could be attributed to missing data points (an excess of missing '+' or '-') and compounded by the small sample size. The exception, (J17) sR1210x, showed fewer *B. carinata* alleles, possibly due to its location near the end of its chromosome. For some loci, the only alleles observed were the *B. napus* alleles ("-") (i.e. sR6083b, data not shown). This constituted extreme segregation distortion, and was due to the absence of *B. carinata* loci near the tips of linkage groups, presumably as a result of either a translocation or loss of a terminal chromosomal segment.

Table 4-1: B genome allele segregation x^2 values for the BC1 mapping population. LG indicates the linkage group to which core markers were mapped in the KCN-5 mapping population. Chi-squared values (x^2) were calculated using a 1:1 expected allele ratio. The *p*-value was calculated for 1 *d.f.* The S column: "ns" indicates "not-significant", while "*" to "****" indicates significant ($p< 0.05$), to highly significant ($p< 0.0001$) deviations from the H_o. Arrows indicated distortion in favor (↑) of *B. carinata* content. J16, J17 and J18 are presented in Appendix A.

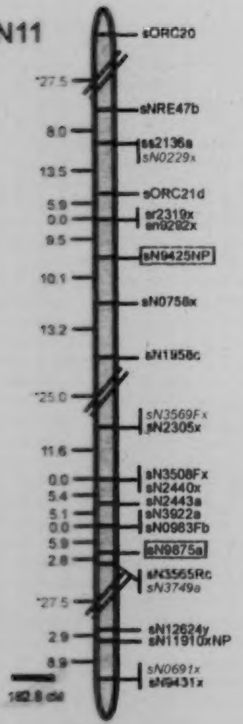
B Genome Linkage Groups					B Genome Linkage Groups				
LG	*Marker*	$\chi^2_{1:1}$	*p*	*S*	*LG*	*Marker*	$\chi^2_{1:1}$	*p*	*S*
J11	sj2013z	3	0.0833	ns	J14	sj2042b	0.25	0.6171	ns
J11	sj3838fx	3.79	0.0516	ns	J14	sj13101y	0.76	0.3841	ns
J11	sn0412b	1.88	0.1701	ns	J14	sb1060Fa	0	1	ns
J11	sn11516y	1.68	0.1944	ns	J14	sb1859Ba	0.03	0.8618	ns
J11	sn9756x	1.78	0.1824	ns	J14	snre30y	0.11	0.7456	ns
J11	sj1822a	1.68	0.1944	ns	J14	sb0273a	0.03	0.8728	ns
J11	sb1789a	2.08	0.1495	ns	J14	sb2972iic	0.29	0.593	ns
J11	sb2510x	2.5	0.1139	ns	J14	sb0334x	0.64	0.4233	ns
J11	ss2210By	1.6	0.2059	ns	J14	sj0927a	0.44	0.505	ns
J11	sb2755x	0.47	0.4927	ns	J14	sj0143z	1.26	0.2623	ns
J11	sn9875w	0.95	0.3304	ns	J14	sb0372a	0.42	0.5164	ns
J11	sj0644a	0.24	0.6219	ns	J14	sj3969x	0.26	0.6121	ns
J11	sb0563ia	0.24	0.6219	ns	J14	sb1060Fia	0.24	0.6219	ns
J11	sn7518y	0.03	0.8575	ns	J14	sb1828	0.11	0.7389	ns
J11	sb2755	0.03	0.8618	ns	J14	sb23194a	0.47	0.4927	ns
J12	sj3503Ra	4.8	0.0285	*↑	J14	sb2108F	0.64	0.4233	ns
J12	sn11670x	10	0.0016	**↑	J14	sb0268	0.13	0.7237	ns
J12	sb1856a	7.53	0.0061	**↑	J14	sb1935Ax	0.24	0.6219	ns
J12	sj3302Ria	3.57	0.0588	ns	J14	sj3969a	0.03	0.8694	ns
J12	sn3888fy	8.1	0.0044	**↑	J15	sb0273w	2.5	0.1139	ns
J12	sb1726x	8.53	0.0035	**↑	J15	sj1071t	4.83	0.028	*↑
J12	sj0397Ry	7.26	0.0071	**↑	J15	sb0580Rx	4.24	0.0396	*↑
J12	sj1158a	8.53	0.0035	**↑	J15	sb3176Fa	3.46	0.063	ns
J12	sb2769b	15	0.0001	***↑	J15	sb2303y	0.31	0.5775	ns
J12	sb0360x	9.26	0.0024	**↑	J15	sj1071w	6.43	0.0112	*↑
J12	sj13101x	7.81	0.0052	**↑	J15	sn0818fx	3.27	0.0706	ns
J12	sn3761x	7.81	0.0052	**↑	J15	sb1817x	0.33	0.5637	ns
J12	sn12508iix	6.08	0.0137	*↑	J15	sb0202ia	5.77	0.0163	*↑
J12	ss1949x	0.76	0.3841	ns	J15	sj0655x	6.13	0.0133	*↑
J12	sb3103a	0.06	0.8084	ns	J15	sb1729a	10.53	0.0012	**↑
J12	sj46102c	16.94	4E-05	****↑	J15	sb2120a	9	0.0027	**↑
J13	sb2543Ra	1.4	0.2367	ns	J15	sb2334x	3.33	0.0679	ns
J13	sb1936Ax	1	0.3173	ns	J15	sn1925x	10.67	0.0011	**↑
J13	sb1936Ay	0.68	0.4111	ns	J15	snrd40x	6.26	0.0124	*↑
J13	sj1322a	1.58	0.2087	ns	J15	sn12118x	8.1	0.0044	**↑
J13	sj03132x	1.48	0.223	ns	J15	sn3761y	6.08	0.0137	*↑
J13	sj3910a	3.27	0.0706	ns	J15	sn4276y	8.76	0.0031	**↑
J13	sj1086x	4.57	0.0326	*↑	J15	sb1992b	6.43	0.0112	*↑
J13	sn12917x	3.46	0.063	ns	J15	sb1956b	15.13	0.0001	***↑
J13	sn8348w	4	0.0455	*↑	J15	snre47x	3.79	0.0516	ns
J13	sn12480x	5.77	0.0163	*↑	J15	sb1538Bx	7.81	0.0052	**↑
J13	sb2668	2.63	0.1048	ns	J15	sr12095x	1	0.3173	ns
J13	sorg95x	3.79	0.0516	ns	J15	sb2365x	2.78	0.0956	ns
J13	snrb35x	2.78	0.0956	ns	J15	sj1071s	0.71	0.398	ns
J13	sb0862a	3.27	0.0706	ns	J15	sj1071y	6.43	0.0112	*↑
J13	sb1752x	1.06	0.3035	ns					
J13	sj1473x	4.17	0.0411	*↑					
J13	sn13083y	0.13	0.715	ns					
J13	snrb45x	1.32	0.2498	ns					

Table 4-2: C genome allele segregation x^2 values for the BC1 mapping population. LG indicates the linkage group to which core markers were mapped in the KCN-5 mapping population. Chi-squared values (x^2) were calculated using a 1:1 expected allele ratio. The *p*-value was calculated for 1 *d.f.* The S column: "ns" indicates "not-significant", while "*" to "****" indicates significant ($p < 0.05$), to highly significant ($p < 0.0001$) deviations from the H_0. Arrows indicated distortion in favor (↑) of *B. carinata* content. N15, N16, N17, N18 and N19 are presented in Appendix A.

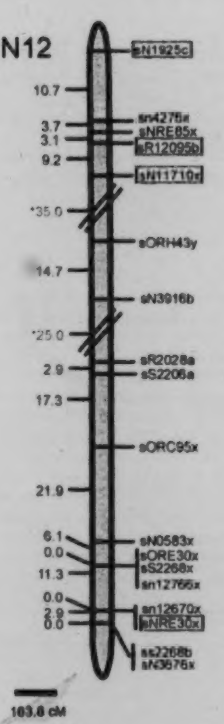
C Genome Linkage Groups					C Genome Linkage Groups				
LG	Marker	$\chi^2_{1:1}$	p	S	LG	Marker	$\chi^2_{1:1}$	p	S
N11	sorc20	1.3	0.2498	ns	N13	sn2034	0	1	ns
N11	snre47b	1.6	0.2059	ns	N13	ss2368a	0.4	0.505	ns
N11	ss2136a	0.2	0.631	ns	N13	sn8956Rt	0.5	0.4795	ns
N11	sn0229x	0.9	0.3458	ns	N13	sr1346a	3.8	0.0516	ns
N11	sorc21d	0.1	0.7518	ns	N13	sore37a	2.5	0.1139	ns
N11	sr2319x	2.6	0.1048	ns	N13	sn1844x	2.5	0.1139	ns
N11	sn9292x	1.1	0.3035	ns	N13	sn8841a	0.1	0.7518	ns
N11	sn9425	4.2	0.0396	*↑	N13	sn3676c	0.5	0.4652	ns
N11	sn0758x	0.7	0.398	ns	N13	sn12574x	0.1	0.7389	ns
N11	sn1958c	2.9	0.0864	ns	N13	sn0583y	0	0.8658	ns
N11	sn2305x	1.7	0.1944	ns	N13	sr2319y	0.1	0.7456	ns
N11	sn2440x	0.3	0.6121	ns	N13	snrb35a	0.4	0.505	ns
N11	sn3508Fx	0.7	0.4111	ns	N13	sn8348a	0	0.8694	ns
N11	sn2443a	0.9	0.3428	ns	N13	sj3969y	1	0.3173	ns
N11	sn0983Fb	0.6	0.4233	ns	N13	sn0704y	3.5	0.063	ns
N11	sn3922a	1.1	0.3035	ns	N13	ss2331Ax	0.2	0.631	ns
N11	sn9875a	0.4	0.505	ns	N13	sorh43x	1	0.3173	ns
N11	sn3565Rc	0.1	0.7389	ns	N13	sb2972iix	0	1	ns
N11	sn12624y	0.4	0.505	ns	N13	sn8115Fa	0.1	0.7389	ns
N11	sn11910x	0	1	ns	N13	sorc21z	0.9	0.3428	ns
N11	sn9431x	0.6	0.4233	ns	N13	sn8956Rw	4.8	0.028	*↑
N12	sn1925c	3.2	0.0736	ns	N13	sorc95b	8.5	0.0035	**↑
N12	sn4276x	1.2	0.2733	ns	N13	sorc76x	0.4	0.5271	ns
N12	snre85x	1.4	0.2367	ns	N13	sn2429x	0.6	0.4233	ns
N12	sr12095b	0.1	0.7389	ns	N13	sorc21b	1.3	0.2623	ns
N12	sn11710x	1.4	0.2367	ns	N13	sr1210a	3.1	0.0782	ns
N12	sorh43y	2	0.1573	ns	N14	sn11859x	0.7	0.4111	ns
N12	sn3916b	3.2	0.0719	ns	N14	sn0584	1.8	0.1824	ns
N12	sn3676x	0.3	0.5775	ns	N14	sorh28a	0	0.8618	ns
N12	ss2268b	0	0.8658	ns	N14	sn0768a	0.1	0.7518	ns
N12	snre30x	0	1	ns	N14	sb2352fx	0.4	0.505	ns
N12	sn12670x	0.1	0.7389	ns	N14	sb1768x	1.1	0.3035	ns
N12	sn12766x	0.2	0.631	ns	N14	sn11516a	0.2	0.6219	ns
N12	ss2268x	0	0.8658	ns	N14	sorg90	0.1	0.7456	ns
N12	sore30x	0.1	0.7389	ns	N14	sn0704a	0.3	0.6121	ns
N12	sn0583x	0.7	0.398	ns	N14	sn0412y	3.7	0.0555	ns
N12	sorc95x	0.1	0.7456	ns	N14	sn3508Fw	4	0.0455	*↓
N12	ss2206a	0.2	0.631	ns	N14	sorh43a	4.8	0.028	*↓
N12	sr2028a	0	0.8658	ns	N14	sn3676y	2.1	0.1441	ns
					N14	sore30y	1.8	0.1824	ns
					N14	ss1852x	1.3	0.2568	ns
					N14	ss2210Bx	3.1	0.0782	ns
					N14	sn12624x	1.8	0.1824	ns
					N14	sn3676z	0.6	0.4497	ns
					N14	sore79y	1.6	0.2059	ns

Figure 4-7: KCN-5 mapping population C genome linkage groups N11-N15. The graphic shows the LGs in terms of RF values (in cM) generated from the KCN-5 mapping population scoring matrix. RF values are shown to the left of the linkage group, while SSR markers are to the right. RF values marked with an asterisk were manually calculated and are not drawn to scale. The total size of each LG according to RF values is noted to the left-bottom of each LG graphic. Markers in grey boxes are publicly available (Lydiate, D., and Sharpe, A., available through material transfer agreement). Italicized markers showed poor amplification in the mapping population but were assigned to a LG with a positive match of the allele with one or more reference maps.

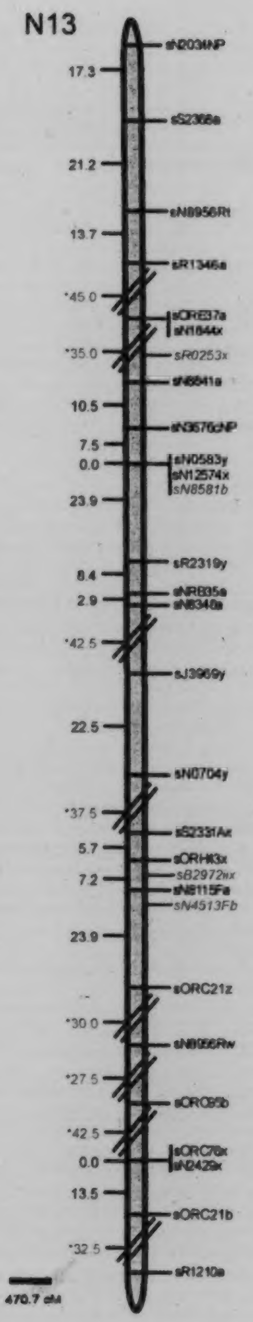
N11


sORC20
*27.5
sNRE47b
8.0
ss2136a
sN0229x
13.5
sORC21d
5.9
0.0
sr2319x
sn0292x
9.5
sN9425NP
10.1
sN0758x
13.2
sN1958c
*25.0
sN3569Fx
sN2305x
11.6
0.0
sN3508Fx
sN2440x
5.4
sN2443a
5.1
sN3922a
0.0
sN0983Fb
5.9
sN9875a
2.8
sN3565Rc
sN3749a
*27.5
sN12624y
2.9
sN11910xNP
8.9
sN0691x
sN9431x
162.8 cM

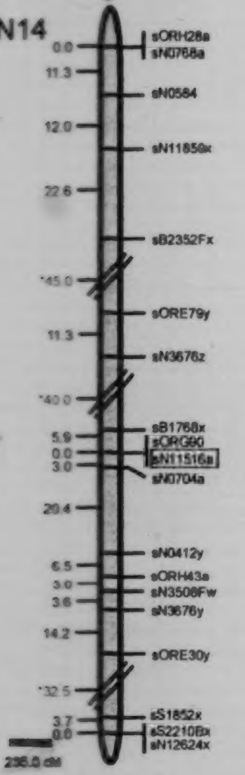
N12


sN1925c
10.7
sn4276x
3.7
sNRE85x
3.1
sR12095b
9.2
sN11710x
*35.0
sORH43y
14.7
sN3916b
*25.0
sR2028a
2.9
sS2206a
17.3
sORC95x
21.9
6.1
sN0583x
0.0
sORE30x
sS2268x
11.3
sn12766x
0.0
sn12670x
2.9
sNRE30x
0.0
ss2268b
sN3676x
103.8 cM

N13


sN2034NP
17.3
sS2368a
21.2
sN8956Rt
13.7
sR1346a
*45.0
sORE37a
sN1844x
*35.0
sR0253x
sN8841a
10.5
sN3676cNP
7.5
sN0583y
0.0
sN12574x
sN8581b
23.9
sR2319y
8.4
sNR835a
2.9
sN8348a
*42.5
sJ3969y
22.5
sN0704y
*37.5
sS2331Ax
5.7
sORH43x
sB2972wx
7.2
sN8115Fa
sN4513Fb
23.9
sORC21z
*30.0
sN8956Rw
*27.5
sORC95b
*42.5
sORC76x
0.0
sN2429x
13.5
sORC21b
*32.5
sR1210a
470.7 cM

N14


sORH28a
0.0
sN0768a
11.3
sN0584
12.0
sN11859x
22.6
sB2352Fx
*45.0
sORE79y
11.3
sN3676z
*40.0
sB1768x
5.9
sORG90
0.0
sN11516a
3.0
sN0704a
20.4
sN0412y
6.5
sORH43a
3.0
sN3508Fw
3.6
sN3676y
14.2
sORE30y
*32.5
sS1852x
3.7
0.0
sS2210Bx
sN12624x
236.0 cM

N15

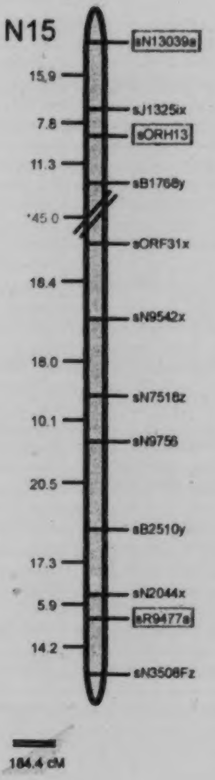
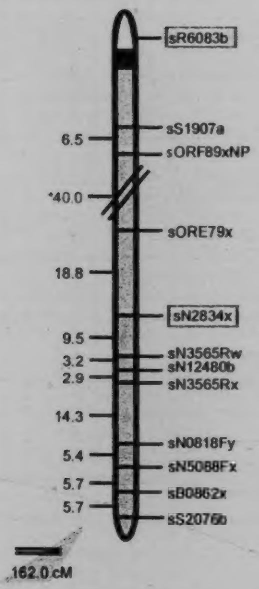
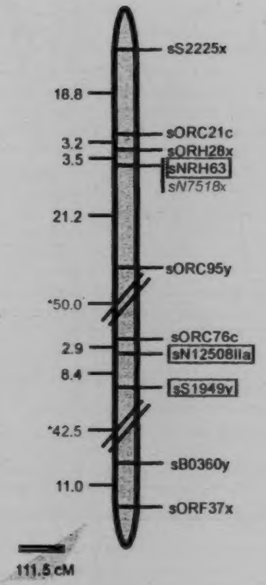

sN13039a
15,9
sJ1325ix
7.8
sORH13
11.3
sB1768y
*45.0
sORF31x
18.4
sN9542x
18.0
sN7518z
10.1
sN9756
20.5
sB2510y
17.3
sN2044x
5.9
sR9477a
14.2
sN3508Fz
184.4 cM

Figure 4-8: KCN-5 mapping population C genome linkage groups N16-N19. The graphic shows the linkage groups in terms of RF values (in cM) generated from the KCN-5 mapping population scoring matrix. RF values are shown to the left of the linkage group, while SSR markers are to the right. RF values marked with an asterisk were manually calculated and are not drawn to scale. The total size of each LG according to RF values is noted to the left-bottom of each LG graphic. Markers in grey boxes are publicly available (Lydiate, D., and Sharpe, A., available through material transfer agreement). Italicized markers showed poor amplification in the mapping population but were assigned to a LG with a positive match of the allele with one or more reference maps.

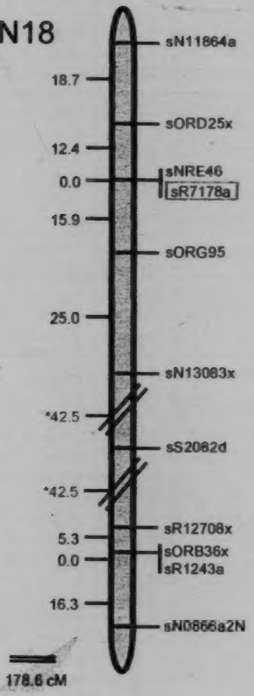
N16


sR6083b
sS1907a
6.5
sORF89xNP
*40.0
sORE79x
18.8
sN2834x
9.5
sN3565Rw
3.2
sN12480b
2.9
sN3565Rx
14.3
sN0818Fy
5.4
sN5088Fx
5.7
sB0862x
5.7
sS2076b
162.0 cM

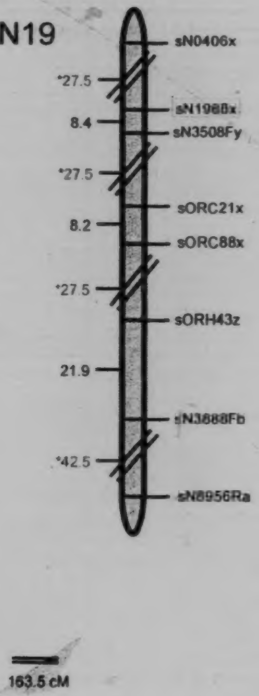
N17


sS2225x
18.8
sORC21c
3.2
sORH28x
3.5
sNRH63
sN7518x
21.2
sORC95y
*50.0
sORC76c
2.9
sN12508IIa
8.4
sS1949y
*42.5
sB0360y
11.0
sORF37x
111.5 cM

N18


sN11864a
18.7
sORD25x
12.4
0.0
sNRE46
sR7178a
15.9
sORG95
25.0
sN13083x
*42.5
sS2082d
*42.5
sR12708x
5.3
0.0
sORB36x
sR1243a
16.3
sN0866a2N
178.6 cM

N19


sN0406x
*27.5
sN1968x
8.4
sN3508Fy
*27.5
sORC21x
8.2
sORC88x
*27.5
sORH43z
21.9
sN3888Fb
*42.5
sN8956Ra
163.5 cM

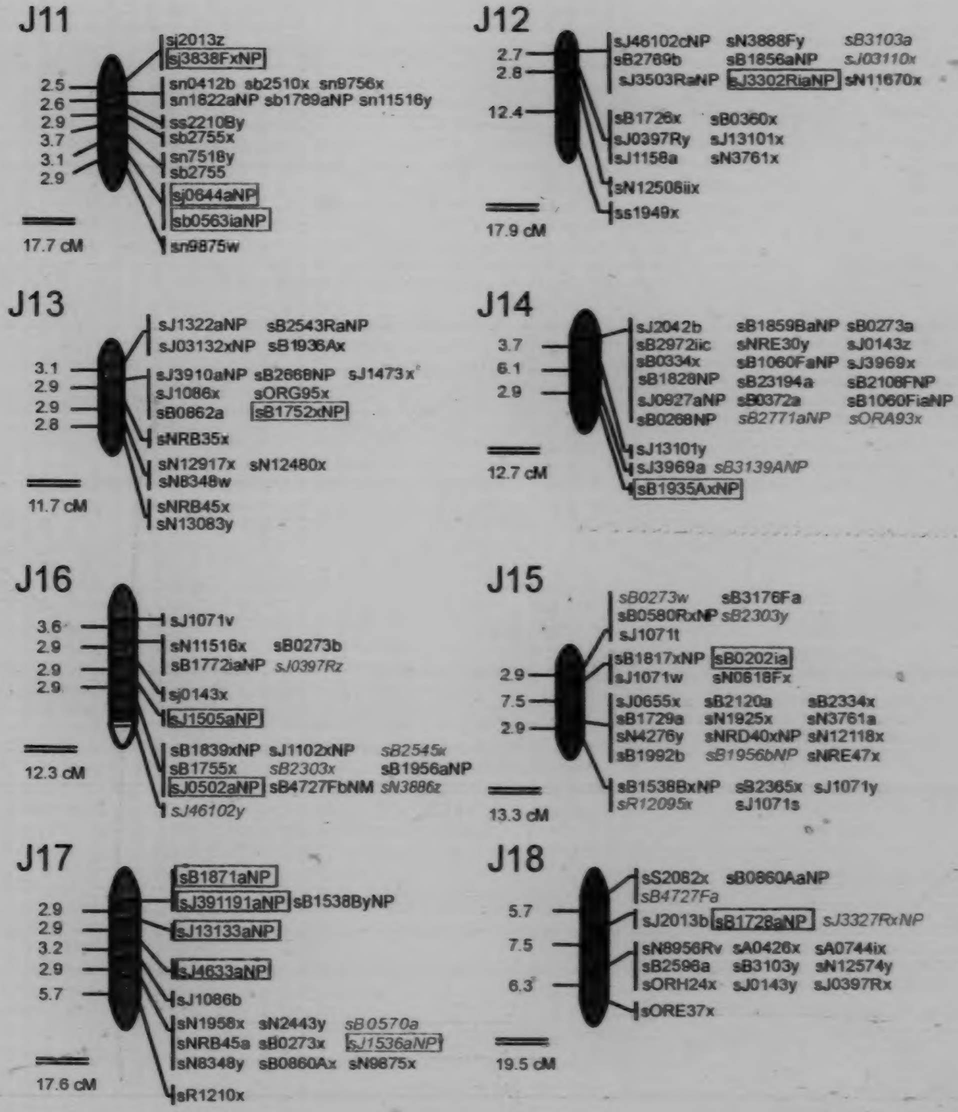

J11
sj2013z
sj3838FxNP
2.5
2.6
2.9
3.7
3.1
2.9
sn0412b
sb2510x
sn9756x
sn1822aNP
sb1789aNP
sn11516y
ss2210By
sb2755x
sn7518y
sb2755
sj0644aNP
sb0563iaNP
sn9875w
17.7 cM
J12
2.7
2.8
12.4
sJ46102cNP
sN3888Fy
sB3103a
sB2769b
sB1856aNP
sJ03110x
sJ3503RaNP
sJ3302RiaNP
sN11670x
sB1726x
sB0360x
sJ0397Ry
sJ13101x
sJ1158a
sN3761x
sN12506iix
ss1949x
17.9 cM
J13
3.1
2.9
2.9
2.8
sJ1322aNP
sB2543RaNP
sJ03132xNP
sB1936Ax
sJ3910aNP
sB2668NP
sJ1473x
sJ1086x
sORG95x
sB0862a
sB1752xNP
sNRB35x
sN12917x
sN12480x
sN8348w
sNRB45x
sN13083y
11.7 cM
J14
3.7
6.1
2.9
sJ2042b
sB1859BaNP
sB0273a
sB2972iic
sNRE30y
sJ0143z
sB0334x
sB1060FaNP
sJ3969x
sB1828NP
sB23194a
sB2106FNP
sJ0927aNP
sB0372a
sB1060FiaNP
sB0268NP
sB2771aNP
sORA93x
sJ13101y
sJ3969a
sB3139ANP
sB1935AxNP
12.7 cM
J16
3.6
2.9
2.9
2.9
sJ1071v
sN11516x
sB0273b
sB1772iaNP
sJ0397Rz
sj0143x
sJ1505aNP
sB1839xNP
sJ1102xNP
sB2545x
sB1755x
sB2303x
sB1956aNP
sJ0502aNP
sB4727FbNM
sN3886z
sJ46102y
12.3 cM
J15
sB0273w
sB3176Fa
sB0580RxNP
sB2303y
sJ1071t
2.9
7.5
2.9
sB1817xNP
sB0202ia
sJ1071w
sN0818Fx
sJ0655x
sB2120a
sB2334x
sB1729a
sN1925x
sN3761a
sN4276y
sNRD40xNP
sN12118x
sB1992b
sB1956bNP
sNRE47x
sB1538BxNP
sB2365x
sJ1071y
sR12095x
sJ1071s
13.3 cM
J17
2.9
2.9
3.2
2.9
5.7
sB1871aNP
sJ391191aNP
sB1538ByNP
sJ13133aNP
sJ4633aNP
sJ1086b
sN1958x
sN2443y
sB0570a
sNRB45a
sB0273x
sJ1536aNP
sN8348y
sB0860Ax
sN9875x
sR1210x
17.6 cM
J18
5.7
7.5
6.3
sS2082x
sB0860AaNP
sB4727Fa
sJ2013b
sB1728aNP
sJ3327RxNP
sN8956Rv
sA0426x
sA0744ix
sB2596a
sB3103y
sN12574y
sORH24x
sJ0143y
sJ0397Rx
sORE37x
19.5 cM

Figure 4-9: KCN-5 mapping population B genome linkage groups. The graphic shows the linkage groups in terms of cM, generated from the KCN-5 mapping population scoring matrix. *p*RF values are shown to the left of each linkage group, while SSR markers are to the right. The total 'size' of each LG according to *p*RF values is noted to the left-bottom of each LG graphic. Markers in grey boxes are publicly available (Lydiate, D., and Sharpe, A., available through material transfer agreement). Italicized markers showed poor amplification in the mapping population but were assigned to the LG with a positive match of the allele with one or more reference maps.

4.4. Graphical genotypes for the KCN-5 mapping population and the KCN-5 Sibling family

The graphical genotypes of the KCN-5 mapping population were primarily constructed using scoring matrix data from the KCN-5 BC1 and BC2 sibling familiy, and corroborated using the allele identities from the AAFC reference populations and screen panels. If the scoring data failed to provide a definitive ordering of loci (i.e. B genome), AAFC reference maps and corresponding allele identities were used to infer the most probable marker orientation (Figures 4-1 and 4-2). These maps provided a visual interpretation of *B. carinata* introgression and were used to evaluate the effects of backcrossing on chromosome inheritance.

The linkage groups J11-J18 (Figures 4-10 to 4-13) and N11, N13 (Figure 4-14) were examined through the construction of graphical genotypes. Following the assembly of the data from the KCN-5 mapping population into 17 graphical chromosomes, the KCN-5 sibling family was assayed with the SSR subset (220 markers - amplifying 344 alleles) previously discussed in section 4.1.5. KCN-5, the F1 progenitor was also assayed in this manner.

Linkage groups N11 and N13 (Figure 4-14) were composed of portions of both parental chromosomes in BC1 plants. In the case of N11, 4 BC1 plants maintained the complete *B. carinata* chromosome, while 2 BC1 plants retained *B. carinata* N13. In contrast, only one plant for each of N11 and N13 had lost all *B. carinata* alleles for these linkage groups. The BC2 patterns of inheritance corresponded to the expected pattern of BC2 *B. carinata* introgressions given the content of the progenitor BC1 plant.

The assayed alleles were also used to determine the amount of *B. carinata* material inherited. For example, the KCN-mapping population N11 chromosome exhibited an average of 12.5 *B. carinata* alleles/24 possible alleles. This was a statistically significant, ($x^2_{1:3}$=7.15 d.f.=1), departure from the expected 1:3 ratio expected at this stage of introgression (Griffiths *et al.*, 2005). Conversely, the BC2 linkage groups presented an average of 3.9 alleles/ chromosome, which was not statistically significant ($x^2_{1:7}$=0.27 d.f.=1) from the expected 3/24 alleles per N11 linkage group. The introgression of N13 followed the same trend.

When allele identities were used to align the B genome loci, it was evident that B genome chromosomes were inherited as whole chromosomes and not as introgressed segments. B genome graphical genotypes reflected the low theoretical RF values obtained earlier, in that the loss of terminal loci was interpreted as recombination by mapping software functions.

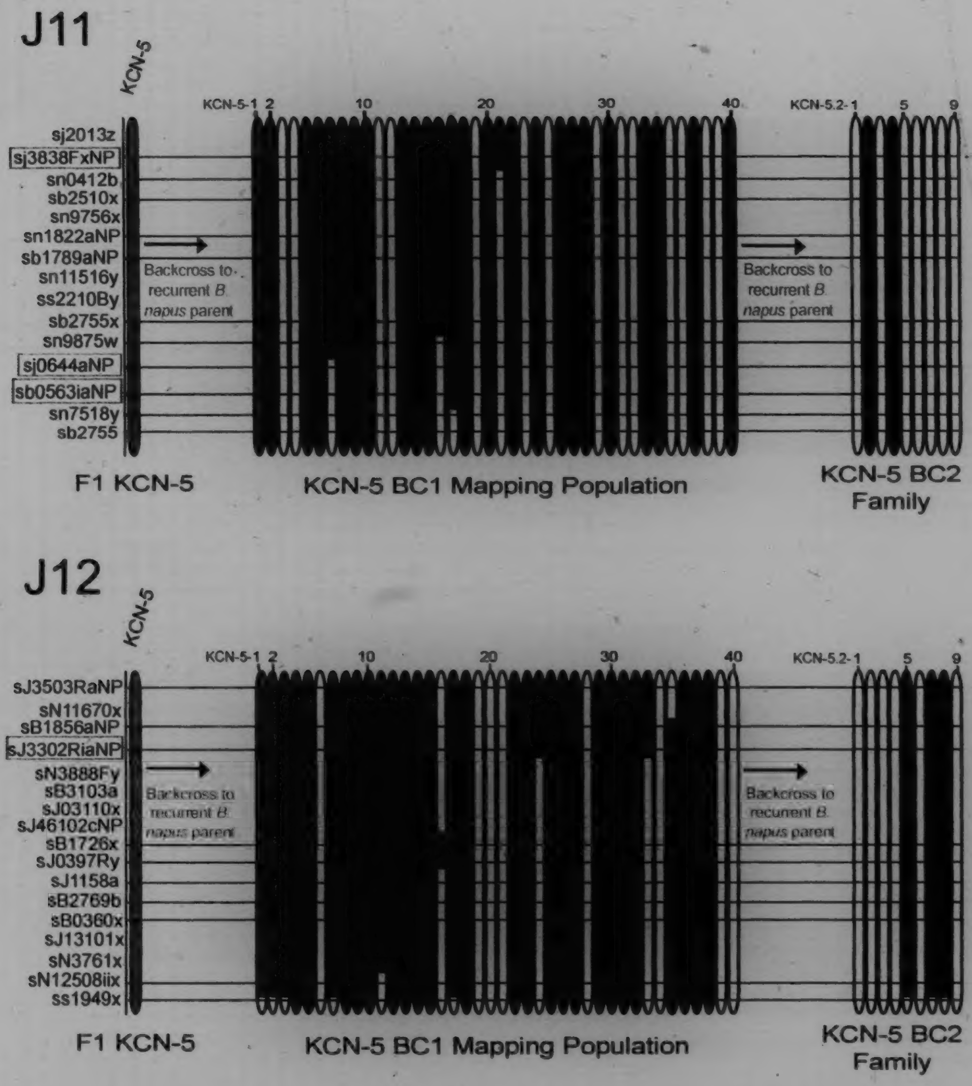

J11
KCN-5
KCN-5-1
2
10
20
30
40
KCN-5.2-1
5
9
sj2013z
sj3838FxNP
sn0412b
sb2510x
sn9756x
sn1822aNP
sb1789aNP
sn11516y
ss2210By
sb2755x
sn9875w
sj0644aNP
sb0563iaNP
sn7518y
sb2755
Backcross to recurrent B. napus parent
Backcross to recurrent B. napus parent
F1 KCN-5
KCN-5 BC1 Mapping Population
KCN-5 BC2 Family
J12
KCN-5
KCN-5-1
2
10
20
30
40
KCN-5.2-1
5
9
sJ3503RaNP
sN11670x
sB1856aNP
sJ3302RiaNP
sN3888Fy
sB3103a
sJ03110x
sJ46102cNP
sB1726x
sJ0397Ry
sJ1158a
sB2769b
sB0360x
sJ13101x
sN3761x
sN12508iix
ss1949x
Backcross to recurrent B. napus parent
Backcross to recurrent B. napus parent
F1 KCN-5
KCN-5 BC1 Mapping Population
KCN-5 BC2 Family

Figure 4-10: KCN-5 mapping population graphical genotype of J11 and J12. The graphical genotype represents the recombination events/allele loss that occurred in the meioses of the 40 F1 hybrid gametes, and 9 BC1 gametes. SSR markers are shown on the left and are distributed along the LG evenly. Horizontal lines from SSR markers were markers that also amplified alleles on this LG in the AAFC reference maps (Lydiate, D. and Sharpe A., *unpublished*). Markers without horizontal lines are novel alleles. The yellow shaded tags highlight the maternal parents to the subsequent family. Shaded sections of the ideogram represent the presence of *B. carinata* loci, while the white areas indicate the absence of *B. carinata* loci. Hatched sections indicate regions between markers where a LG disruption or a recombination event occurred. Where a hatched section spans a marker, the hatching indicates a region of failed PCR reaction(s) where recombination/allele loss could have occurred, but could not be monitored.

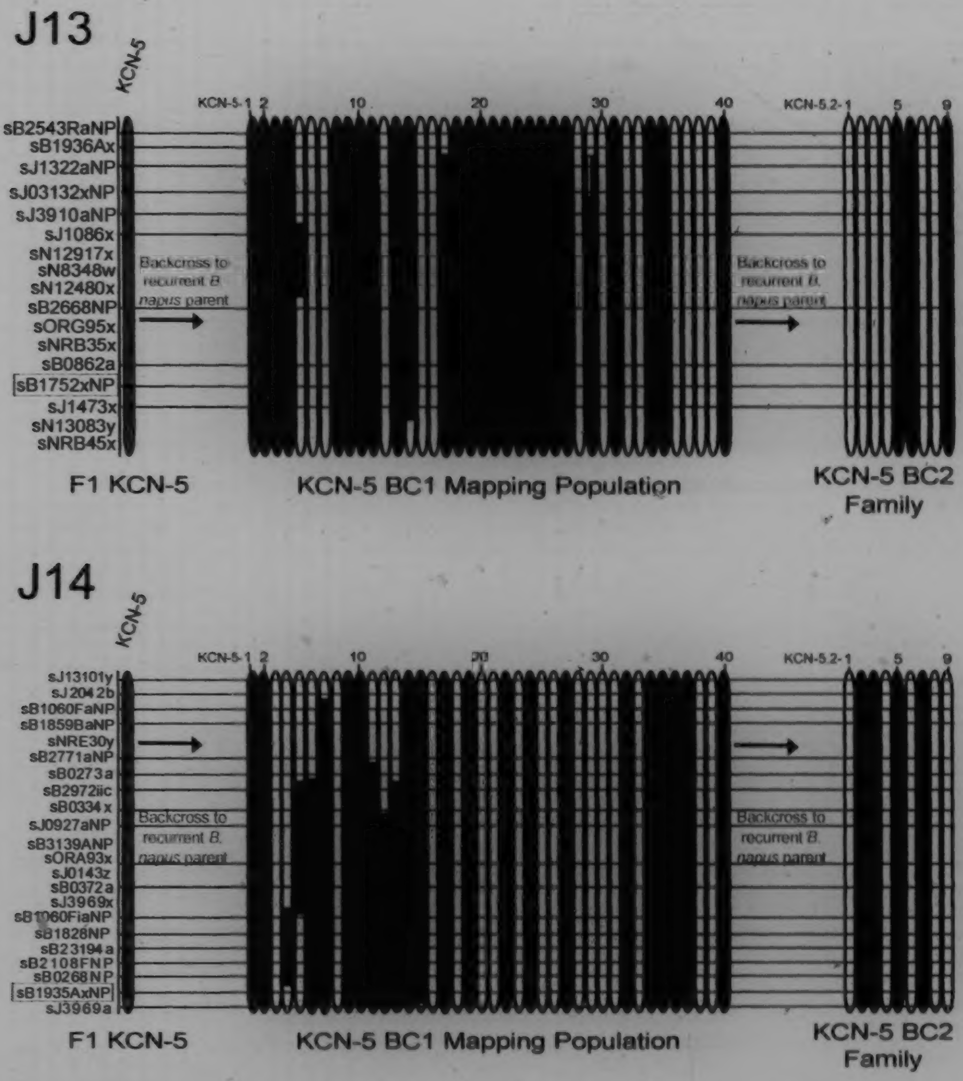

J13
KCN-5
KCN-5-1
2
10
20
30
40
KCN-5.2-1
5
9
sB2543RaNP
sB1936Ax
sJ1322aNP
sJ03132xNP
sJ3910aNP
sJ1086x
sN12917x
sN8348w
sN12480x
sB2668NP
sORG95x
sNRB35x
sB0862a
sB1752xNP
sJ1473x
sN13083y
sNRB45x
Backcross to recurrent B. napus parent
Backcross to recurrent B. napus parent
F1 KCN-5
KCN-5 BC1 Mapping Population
KCN-5 BC2 Family
J14
KCN-5
KCN-5-1
2
10
20
30
40
KCN-5.2-1
5
9
sJ13101y
sJ2042b
sB1060FaNP
sB1859BaNP
sNRE30y
sB2771aNP
sB0273a
sB2972iic
sB0334x
sJ0927aNP
sB3139ANP
sORA93x
sJ0143z
sB0372a
sJ3969x
sB1060FiaNP
sB1828NP
sB23194a
sB2108FNP
sB0268NP
sB1935AxNP
sJ3969a
Backcross to recurrent B. napus parent
Backcross to recurrent B. napus parent
F1 KCN-5
KCN-5 BC1 Mapping Population
KCN-5 BC2 Family

Figure 4-11: KCN-5 mapping population graphical genotype of J13 and J14. The graphical genotype represents the recombination events/ allele loss that occurred in the meioses of the 40 F1 hybrid gametes, and 9 BC1 gametes. SSR markers are shown on the left and are distributed along the LG evenly. Horizontal lines from SSR markers were markers that also amplified alleles on this LG in the AAFC reference maps (Lydiate, D. and Sharpe A., *unpublished*). Markers without horizontal lines are novel alleles. The yellow shaded tags highlight the maternal parents to the subsequent family. Shaded sections of the ideogram represent the presence of *B. carinata* loci, while the white areas indicate the absence of *B. carinata* loci. Hatched sections indicate regions between markers where a LG disruption or a recombination event occurred. Where a hatched section spans a marker, the hatching indicates a region of failed PCR reaction(s) where recombination/ allele loss could have occurred, but could not be monitored.

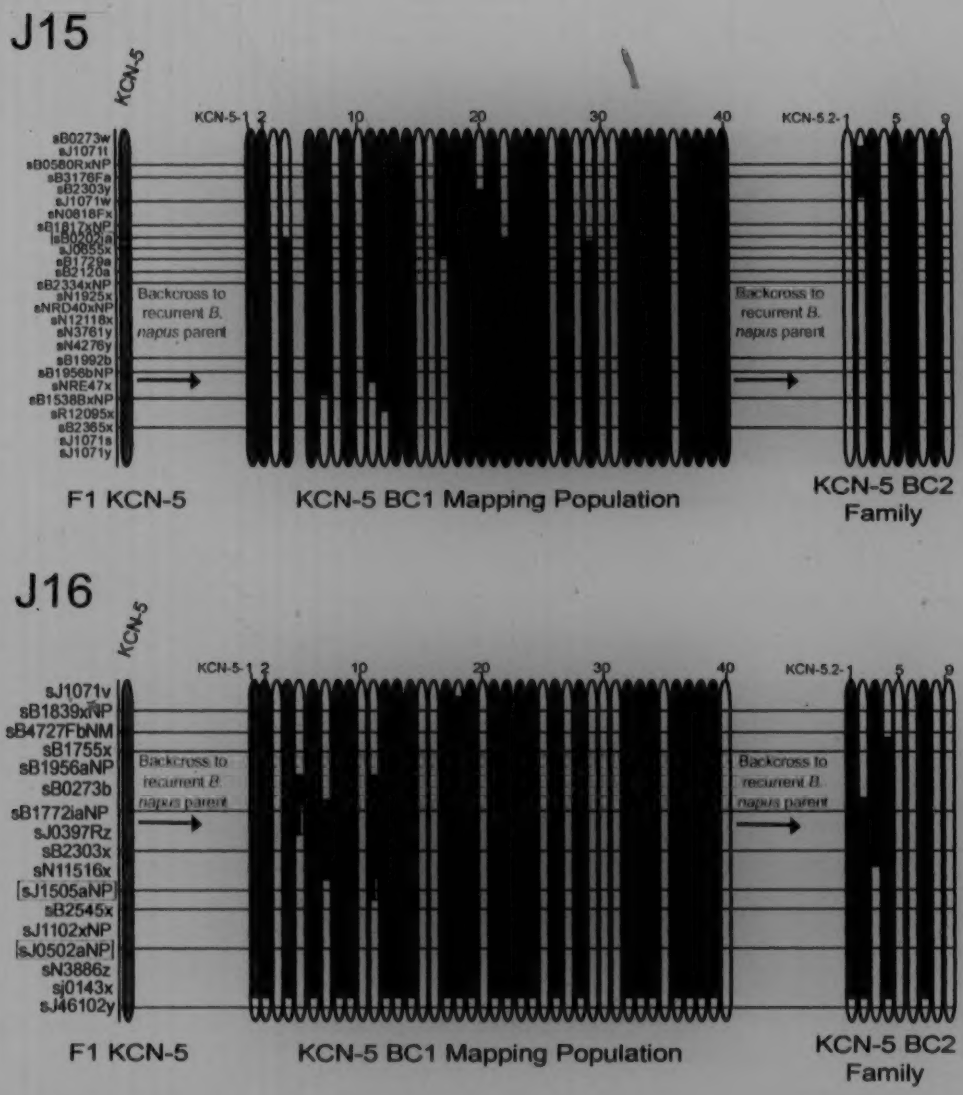

J15
KCN-5
KCN-5-1 2
10
20
30
40
KCN-5.2-1
5
9
sB0273w
sJ1071t
sB0580RxNP
sB3176Fa
sB2303y
sJ1071w
sN0818Fx
sB1817xNP
sB0202ia
sJ0655x
sB1729a
sB2120a
sB2334xNP
sN1925x
sNRD40xNP
sN12118x
sN3761y
sN4276y
sB1992b
sB1956bNP
sNRE47x
sB1538BxNP
sR12095x
sB2365x
sJ1071s
sJ1071y
Backcross to recurrent B. napus parent
Backcross to recurrent B. napus parent
F1 KCN-5
KCN-5 BC1 Mapping Population
KCN-5 BC2 Family
J16
KCN-5
KCN-5-1 2
10
20
30
40
KCN-5.2-1
5
9
sJ1071v
sB1839xNP
sB4727FbNM
sB1755x
sB1956aNP
sB0273b
sB1772iaNP
sJ0397Rz
sB2303x
sN11516x
sJ1505aNP
sB2545x
sJ1102xNP
sJ0502aNP
sN3886z
sj0143x
sJ46102y
Backcross to recurrent B. napus parent
Backcross to recurrent B. napus parent
F1 KCN-5
KCN-5 BC1 Mapping Population
KCN-5 BC2 Family

Figure 4-12: KCN-5 mapping population graphical genotype of J15 and J16. The graphical genotype represents the recombination events/ allele loss that occurred in the meioses of the 40 F1 hybrid gametes, and 9 BC1-gametes. SSR markers are shown on the left and are distributed along the LG evenly. Horizontal lines from SSR markers were markers that also amplified alleles on this LG in the AAFC reference maps (Lydiate, D. and Sharpe A., *unpublished*). Markers without horizontal lines are novel alleles. The yellow shaded tags highlight the maternal parents to the subsequent family. Shaded sections of the ideogram represent the presence of *B. carinata* loci, while the white areas indicate the absence of *B. carinata* loci. Hatched sections indicate regions between markers where a LG disruption or a recombination event occurred. Where a hatched section spans a marker, the hatching indicates a region of failed PCR reaction(s) where recombination/ allele loss could have occurred, but could not be monitored.

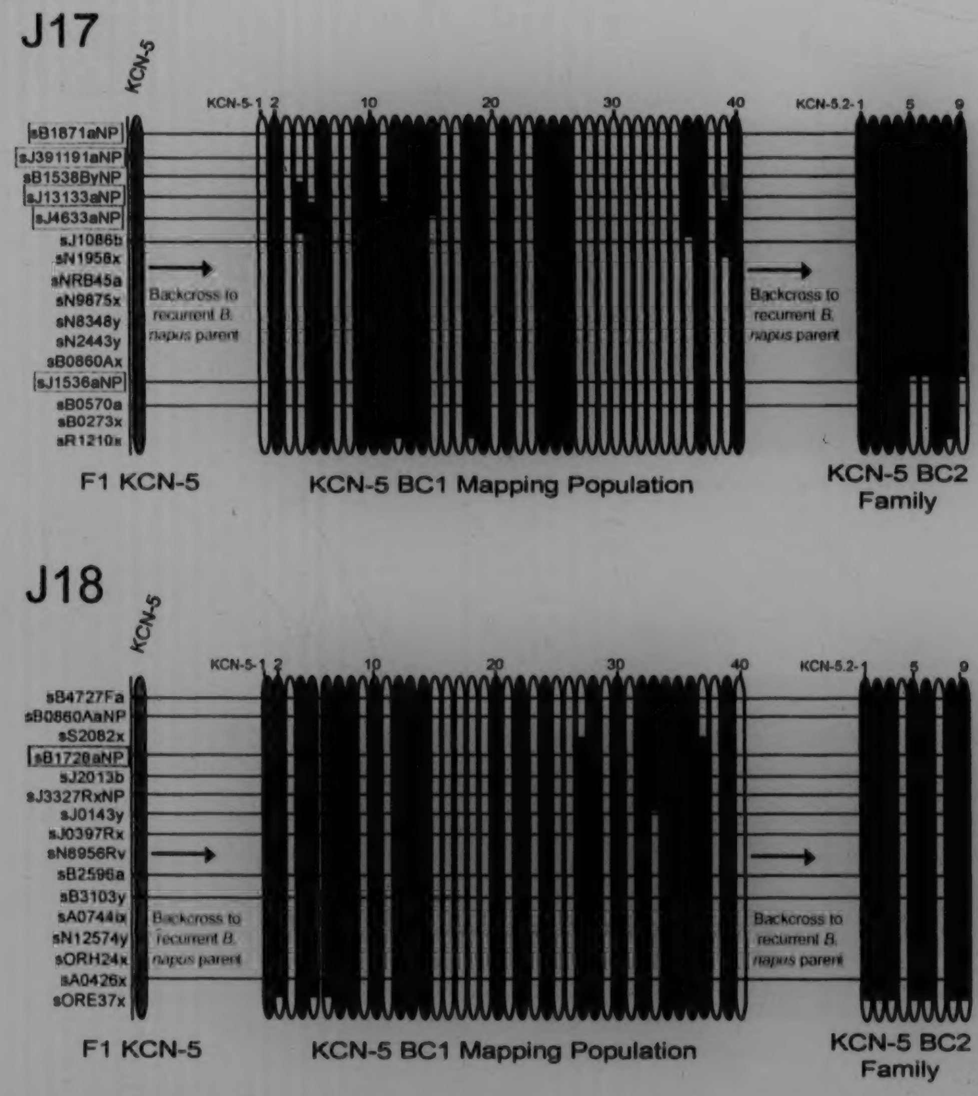

J17
KCN-5
KCN-5-1
2
10
20
30
40
KCN-5.2-1
5
9
sB1871aNP
sJ391191aNP
sB1538ByNP
sJ13133aNP
sJ4633aNP
sJ1086b
sN1958x
sNRB45a
sN9875x
sN8348y
sN2443y
sB0860Ax
sJ1536aNP
sB0570a
sB0273x
sR1210x
Backcross to recurrent B. napus parent
Backcross to recurrent B. napus parent
F1 KCN-5
KCN-5 BC1 Mapping Population
KCN-5 BC2 Family
J18
KCN-5
KCN-5-1
2
10
20
30
40
KCN-5.2-1
5
9
sB4727Fa
sB0860AaNP
sS2082x
sB1728aNP
sJ2013b
sJ3327RxNP
sJ0143y
sJ0397Rx
sN8956Rv
sB2596a
sB3103y
sA0744bx
sN12574y
sORH24x
sA0426x
sORE37x
Backcross to recurrent B. napus parent
Backcross to recurrent B. napus parent
F1 KCN-5
KCN-5 BC1 Mapping Population
KCN-5 BC2 Family

Figure 4-13: KCN-5 mapping population graphical genotype of J17 and J18. The graphical genotype represents the recombination events/allele loss that occurred in the meioses of the 40 F1 hybrid gametes, and 9 BC1 gametes. SSR markers are shown on the left and are distributed along the LG evenly. Horizontal lines from SSR markers were markers that also amplified alleles on this LG in the AAFC reference maps (Lydiate, D. and Sharpe A., *unpublished*). Markers without horizontal lines are novel alleles. The yellow shaded tags highlight the maternal parents to the subsequent family. Shaded sections of the ideogram represent the presence of *B. carinata*loci, while the white areas indicate the absence of *B. carinata* loci. Hatched sections indicate regions between markers where a LG disruption or a recombination event occurred. Where a hatched section spans a marker, the hatching indicates a region of failed PCR reaction(s) where recombination/ allele loss could have occurred, but could not be monitored.

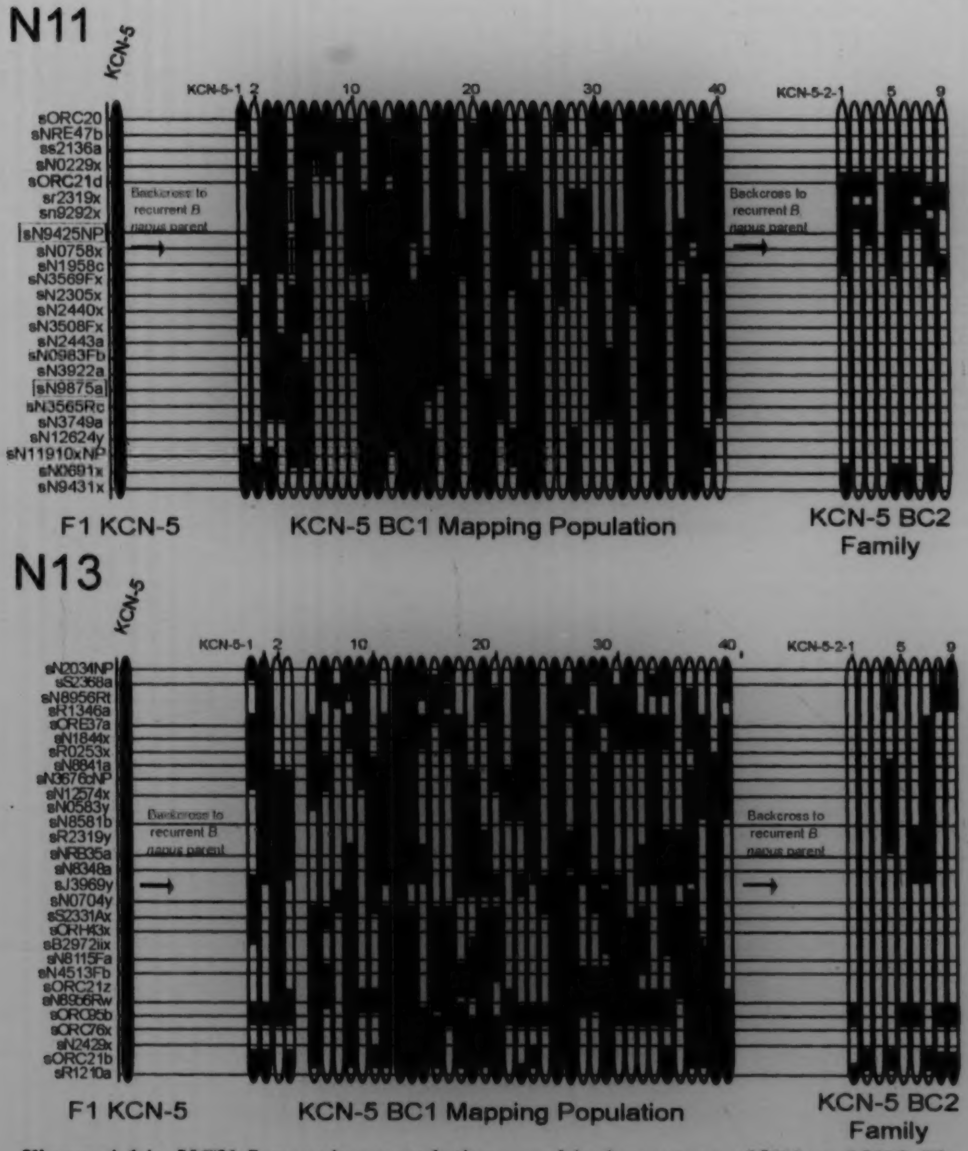


Figure 4-14: KCN-5 mapping population graphical genotype of N11 and N13. The graphical genotype represents the recombination events/allele loss that occurred in the meioses of the 40 F1 hybrid gametes, and 9 BC1 gametes. SSR markers are shown on the left and are distributed along the LG evenly. Horizontal lines from SSR markers were markers that also amplified alleles on this LG in the AAFC reference maps (Lydiate, D. and Sharpe A., *unpublished*). Markers without horizontal lines are novel alleles. The yellow shaded tags highlight the maternal parents to the subsequent family. Shaded sections of the ideogram represent the presence of *B. carinata*loci, while the white areas indicate the absence of *B. carinata* loci. Hatched sections indicate regions between markers where a LG disruption or a recombination event occurred. Where a hatched section spans a marker, the hatching indicates a region of failed PCR reaction(s) where recombination/ allele loss could have occurred, but could not be monitored.

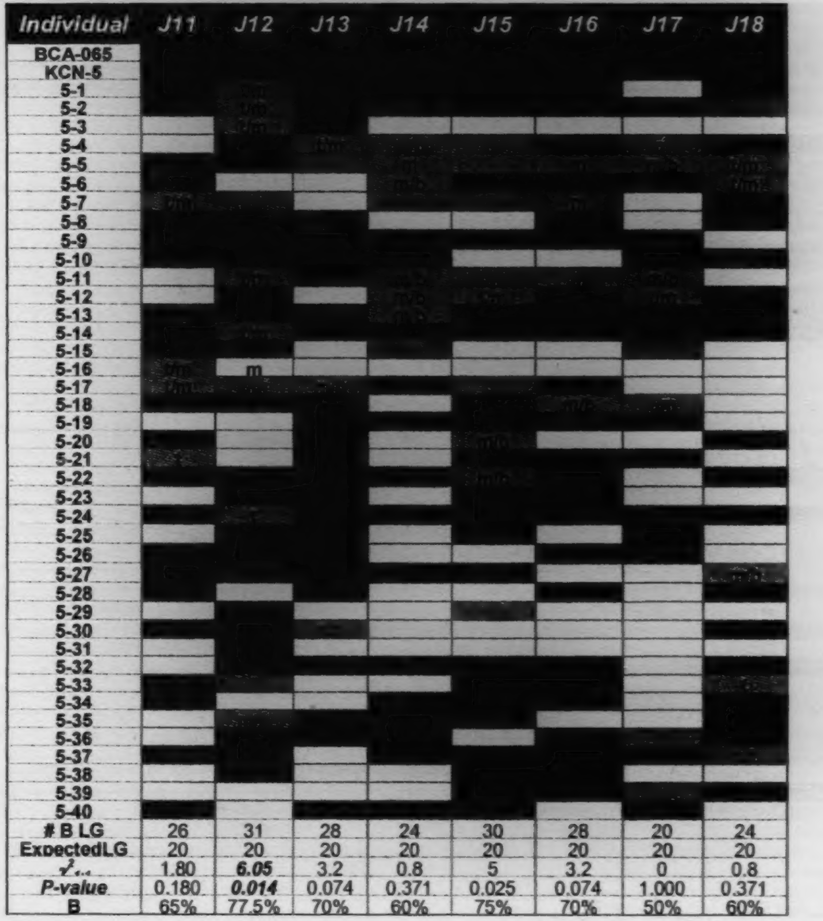
The partially inherited LGs (Figures 4-10 to 4-13) could be interpreted to have lost partial or complete terminal arms, while the centromeric regions (or relic centromeric regions) were maintained. In two cases (Table 4-3 and 4-4), the inherited alleles could not be justified as consisting of conserved portions of the chromosome, these are shaded in yellow. Therefore, when the ratios of inherited LGs were considered, all LGs inheriting their central portions were included (Tables 4-3 and 4-4). If this assumption was not made, the probability of inheriting any complete B genome LG was still high; ranging from 32.5% (J17) to 60% (J16) with an average of 50% for all LG. However, the probability of inheriting no B genome alleles along a LG ranged from 17.5% (J14) to 47.5% (J17), with an average probability of 30%. When all of the progeny was considered, the probability of inheriting each B genome LG ranged from 50% (J17) to 77.5% (J12), with an average probability of 66% for all B genome LGs. Considered separately, only J12 was inherited significantly more often ($\chi^2_{1:1} = 6.05$, *p-value*= 0.014), but overall the B genome was inherited more often than would be expected ($\chi^2_{1:1} = 20.85$, *p-value*= 0.004, d.f.= 7). Non-recombining B genome chromosomes were therefore not preferentially excluded from backcrossed generations. Of the incomplete segments, there were only nine instances of BC1 plants inheriting a portion (5 markers or less) of any B genome chromosome. For the BC2 plants (Table 4-4), there was one incidence of a plant inheriting a similar portion of a B genome linkage group from its progenitor.

Individuals that inherited partial B genome linkage groups with missing terminal loci still showed no evidence of B genome introgression into the A or C genome. Only *B. napus*/ *B. carinata* C genome homologous recombination was evident. It has been previously recognized that the A and C genomes of *Brassica* can undergo homeologous recombination and non-reciprocal translocations (Osborne *et al.*, 2003; Nicolas *et al.*, 2007). As the germplasm was backcross material, A genome bands were monomorphic. Therefore, A genome-linked *B. carinata* loci, originally belonging to the C or B genomes, would appear unlinked. In the KCN-5 mapping population, there was no indication of B genome loci introgressing into the A genome by either recombination or non-reciprocal translocation. Unassigned B genome alleles (Appendix A) were not positively identified as B genome loci by allele identity, and all identified B genome loci were tightly linked by chromosome in the BC1 generation (Figure 4-9). In the KCN-5 BC2 generation, only one example of a possible translocation was apparent (Figure 4-12, J15, KCN-5-2-2), but could not be distinguished from a truncated J15 chromosome.

For the C genome, a non-reciprocal translocation to the A genome would appear as an unlinked section. Several unlinked sections were apparent in the C genome linkage maps; however, a translocation/ non-linkage distinction was not possible when surveying one generation (Figures 4-7 and 4-8). The KCN-5 BC2 family showed no evidence of A/C homeologous recombination or translocation. Additionally, a significant number of C genome SSR primer-pairs were co-dominant, as such; segregating C genome loci from *B. napus* and *B. carinata* were detectable in the experimental germplasm (data not shown).

Individuals in BC1 mapping population frequently inherited multiple B genome LGs (Table 4-3). Five KCN-5 BC1 individuals inherited all eight B genome LGs, while only one individual inherited one LG, and none failed to inherit a single B genome LG. The average was 5.28 LGs per BC1 individual. KCN-5-2 contained all of the B genome conserved regions, and a similar trend of B genome inheritance was observed for the BC2 progeny (Table 4-4). The inheritance ranged from 3 to 6 LGs per BC2 plant, with an average of 4.11 LGs. J16 and J17 showed the loss of additional terminal alleles, otherwise the LGs were conserved from their progenitor. The expected rate of B LG inheritance was not significantly different from an expected 1:1 Mendelian inheritance ratio ($\chi^2_{1:1}$ = 8.44, *p-value*= 0.295, d.f.= 7), however J17 exhibited a higher rate of inheritance. Still, the KCN-5 Sibling population was small for statistical analysis, and while the BC1 population comprised all BC1 seed germinated from a particular cross, the BC2 assayed progeny were selected based on resistance to *L. maculans*. Hence, some bias concerning B genome LG maintenance could have occurred.

Table 4-3: B genome content of the KCN-5 mapping population. Individuals comprising the KCN-5 mapping population and their progenitors were aligned on the vertical axis, while the B genome linkage groups (LGs) were aligned along the top of the table. Darkly shaded boxes indicate the presence of whole LGs, lightly shaded boxes indicate the absence of LGs, and the midtone boxes represent LGs that were present, but missing one or more terminal segment(s). The segments that were present are denoted by: t=top of the LG, m=middle and b=bottom, where 't/m' would indicate that the top and middle of the linkage group were present. The % B Genome indicates the percentage of B linkage groups present in complete or partial form. The $\chi^2_{1:1}$ values, and associated *P-value*, for the presence of each B genome LG shown at the bottom of the table.



Individual	*J11*	*J12*	*J13*	*J14*	*J15*	*J16*	*J17*	*J18*
BCA-065	■	■	■	■	■	■	■	■
KCN-5	■	■	■	■	■	■	■	■
5-1	■	■	■	■	■	■	□	■
5-2	■	■	■	■	■	■	■	■
5-3	□	■	■	□	□	□	□	□
5-4	□	■	■	■	■	■	■	■
5-5	■	■	■	■	■	■	■	■
5-6	■	□	□	■	■	■	■	■
5-7	■	■	□	■	■	■	□	■
5-8	■	■	■	□	□	■	□	■
5-9	■	■	■	■	■	■	■	□
5-10	■	■	■	■	□	□	■	■
5-11	□	■	■	■	■	■	■	□
5-12	□	■	□	■	■	■	■	■
5-13	■	■	■	■	■	■	■	■
5-14	■	■	■	■	■	■	■	■
5-15	■	■	□	■	□	□	■	□
5-16	■	m	□	□	□	□	□	□
5-17	■	■	■	■	■	■	□	□
5-18	■	■	■	□	■	■	■	□
5-19	□	□	■	■	■	■	■	□
5-20	■	□	■	□	■	□	□	■
5-21	■	□	■	□	■	■	■	□
5-22	■	■	■	■	■	■	□	■
5-23	□	■	■	□	■	■	□	□
5-24	■	■	■	■	■	■	■	■
5-25	□	■	■	□	■	□	■	□
5-26	■	■	■	□	□	■	■	□
5-27	■	■	■	■	■	□	□	■
5-28	■	□	■	□	□	■	□	■
5-29	□	■	□	□	■	□	□	□
5-30	■	■	■	□	□	□	□	■
5-31	□	■	□	□	□	□	□	□
5-32	□	■	■	■	■	■	□	■
5-33	■	■	□	□	■	■	□	■
5-34	■	□	□	■	■	■	□	■
5-35	□	■	■	■	■	□	□	■
5-36	□	■	■	■	□	■	■	■
5-37	■	■	□	■	■	■	■	■
5-38	□	■	□	□	■	■	□	□
5-39	□	□	□	□	■	■	■	■
5-40	■	□	■	■	■	□	■	□
# B LG	26	31	28	24	30	28	20	24
ExpectedLG	20	20	20	20	20	20	20	20
$\chi^2_{1:1}$	1.80	***6.05***	3.2	0.8	5	3.2	0	0.8
P-value	0.180	***0.014***	0.074	0.371	0.025	0.074	1.000	0.371
B	65%	77.5%	70%	60%	75%	70%	50%	60%

Table 4-4: B Genome content of the KCN-5 BC2 sibling family. Individuals comprising the BC2 sibling group were aligned on the vertical axis, while the B genome linkage groups (LGs) were aligned along the top of the table. Darkly shaded boxes indicate the presence of the same length of a LG present in KCN-5-2, lightly shaded boxes indicate the absence of LGs, and the midtone boxes represent LGs that were present, but missing one or more terminal segment(s) more than the content of KCN-5-2. The segments that were present are denoted by: t=top of the LG, m=middle and b=bottom, where 't/m' would indicate that the top and middle of the linkage group were present. The % B Genome indicates the percentage of B linkage groups that were present in complete or partial form. The $\chi^2_{1:1}$ values, and associated *P-value*, for the presence of each B genome LG are shown at the bottom of the table.

Individual	*J11*	*J12*	*J13*	*J14*	*J15*	*J16*	*J17*	*J18*	*B Genome LG*
5-2-1	□	□	□	□	□	■	■	■	37.5%
5-2-2	■	□	□	■	t	■	■	■	62.5%
5-2-3	□	□	□	■	■	■	■	■	62.5%
5-2-4	■	□	□	□	□	■	■	□	37.5%
5-2-5	□	■	■	■	■	□	■	■	75%
5-2-6	□	□	■	□	■	□	■	■	50%
5-2-7	□	■	□	■	□	■	■	□	50%
5-2-8	□	■	□	□	■	□	■	■	50%
5-2-9	□	□	■	□	□	□	■	■	37.5%
# B LG	2	3	3	4	4	5	9	7	
# Expected LG	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	
$\chi^2_{1:1}$	1.39	0.50	0.50	0.06	0.06	0.06	***4.50***	1.39	
P-value	0.239	0.480	0.480	0.814	0.814	0.814	***0.034***	0.239	
B Genome LG	22%	33%	33%	44%	44%	56%	100%	78%	

4.5. Comparative recombination frequencies of the C and B genomes

The lack of recombination observed for the KCN-5 mapping population B genome chromosomes in this interspecific cross has not been observed in intraspecific B genome maps derived from *B. juncea* (Christianson *et al.* 2006; Axelsson *et al.* 2000; Lagercrantz and Lydiate 1995). Also, KCN-5 mapping population C genome linkage groups exhibited higher recombination rates than intraspecific *B. napus* maps (Parkin *et al.*, 1997; and Piquemal *et al.*, 2005; Sun *et al.*, 2007). To resolve the differences in recombination between the B and C genome chromosomes involved in an interspecific cross (KCN-5 mapping population) versus the same genomes involved in an intraspecific cross, recombination was contrasted between the two other relevant maps.

Of the characterized mapping populations involving C and B genome chromosomes, the T-1 and 61-9 populations were chosen as they were populations that shared the most alleles in common with the KCN-5 mapping population (Axellson *et al.*, 2000; Parkin *et al.*, 1997). The T-1 map was derived from an interspecific *B. juncea* population, however to date; no *B. carinata* maps have been published. An existing unpublished *B. carinata* B genome map exhibits comparable recombination to the B genome of the Axellson *et al.*, (2000) and Christionson *et al.*, (2006) *B. juncea* maps (Lydiate, D. *unpublished*). A total of 68 common alleles were found between the B genome linkage groups of The T-1 map and the KCN-5 map. Similarly, the C genome linkage groups were contrasted with alleles from the 61-9 *B. napus* population.

Figures 4-14 to 4-16 represent the comparative reduced linkage maps for the B genome contrasting the recombination derived from the T-1 population, with the *p*RF obtained from the KCN-5 mapping population. Likewise, Figure 4-17 represents the same evaluation of three representative C genome linkage groups (N11, N13 and N14).

The proportion of the KCN-5 population *p*RF values, over the T-1 RF values, were calculated. These B genome proportions ranged from 0.026 to 0.183, with an average of 0.137. For example, KCN-5 J11 showed 0.183 of the *p*RF compared to J11 involved in intraspecific meioses. However, the calculated *p*RF (17.7 cM including all mapped alleles) could be attributed to the loss of the terminal markers on the linkage group. These terminal alleles were not inherited in other siblings, indicating a higher probability of allele loss over recombination or translocation (Figure 4-10).

In contrast to the B genome data, the KCN-5 C genome RF values were higher than their chromosome counterparts involved in the conventional 61-9 mapping population (Figure 4-17). Linkage groups N11, N13 and N14 are illustrated, but C genome groups N12, N15-N19, showed the same patterns of heightened recombination (data not shown). The RF of the KCN-5 population N11 was 1.78 fold higher than that of the traditional cross. Likewise, KCN-5 N13 was

2.94 fold higher, and N14 was 2.45 fold higher. Considering *p*RF values of the B genome T-1 population were an average of 12.20 fold higher than the KCN-5 population B genome, the two genomes illustrated vastly different recombination dynamics.

The statistical significance of the recombination differences between the KCN-5 mapping population and the intraspecific AAFC reference populations was ascertained by way of two-proportion *Z*-tests. Table 4-5 shows the resulting Z_{calc} statistics, and the associated *p*-values. One section in the B genome and three sections in the C genome were not significantly different, however, region one on J14 (J14 R1) included only co-mapping alleles, and N11 R1 region was small. Nevertheless, all of the linkage groups showed highly significant deviations from the null-hypothesis (H_0= the two proportions are not significantly different).

Table 4-5: **Two-proportion *Z*-tests.** The table shows *Z*-tests comparing the KCN-5 mapping population recombination to the AAFC reference population recombination (T-1 and 61-9). LG indicated the linkage group, p_1 and p_2 indicated the linkage group proportions, and n_1 and n_2 were the population sizes. The resulting Z_{calc} values and the determinations of significance are shown. The S column: "ns" indicated "not-significant", while "*" to "****" indicated significant ($p < 0.05$), to highly significant ($p < 0.0001$) deviations from the H_0. Arrows indicated KCN-5 population distortion in favor (↑) of recombination, while (↓) indicated lower frequencies of interspecific recombination.

Region	P1	P2	Pop 1	Pop 2	Z_{calc}	P-value	Sig.
J11 R1	0.3	0.029	30	40	6.44	0.0000000	*****↓
J11 R2	0.069	0	30	40	3.41	0.0006548	***↓
J11 R3	0.26	0.086	30	40	3.97	0.0000733	****↓
J12	1.055	0.027	30	40	17.24	0.0000000	*****↓
J13 R1	0.27	0.031	30	40	5.88	0.0000000	*****↓
J13 R2	0.442	0	30	40	9.44	0.0000000	*****↓
J14 R1	0	0	30	40	0	1.0000000	ns
J14 R2	0.699	0.127	30	40	9.90	0.0000000	*****↓
J15	0.95	0.133	30	40	13.68	0.0000000	*****↓
J16 R1	0.238	0	30	40	6.58	0.0000000	*****↓
J16 R2	0.318	0.087	30	40	4.97	0.0000007	*****↓
J17	0.493	0.09	30	40	7.66	0.0000000	*****↓
J18 R1	0.233	0.057	30	40	4.34	0.0000140	****↓
J18 R2	0.433	0.075	30	40	7.13	0.0000000	*****↓
J18 R3	0.072	0	30	40	3.48	0.0004950	***↓
N11 R1	0.267	0.275	30	40	0.15	0.8803792	ns
N11 R2*	0.4405	0.8855	30	40	8.09	0.0000000	*****↑
N13 R1	0.044	0.548	30	40	8.95	0.0000000	*****↑
N13 R2	0.329	0.484	30	40	2.63	0.0085479	**↑
N13 R3*	0.2975	0.9065	30	40	10.65	0.0000000	*****↑
N14 R1	0.155	0.233	30	40	1.63	0.1025109	ns
N14 R2	0.075	0.5	30	40	7.64	0.0000000	*****↑
N14 R3	0.194	0.113	30	40	1.91	0.0561350	ns
N14 R4	0.154	0.734	30	40	9.71	0.0000000	*****↑
N14 R5	0.234	0.412	30	40	3.15	0.0016127	**↑

4.6. Summary and novel findings

Following the development of a BC1 mapping population and a BC2 family, SSR markers were deliberately chosen from pools of characterized markers for the purpose of generating the maximum amount of saturation and informative scoring data per marker used. These markers were chosen based on their ability to distinguish the C and B genomes as well as individual linkage groups. A subset of these markers was also used to assay the F1 progenitor and a subsequent BC2 family for the presence or absence of the characterized alleles.

In *B. carinata/B. napus* interspecific crosses at the BC1 stage, it was determined that B genome homeologous recombination or translocation between the

A or C genomes of *B. napus* was not evident. Conversely, *B.carinata* C genome loci were introgressed into the *B. napus* C genome by homologous recombination. The rate of C genome recombination was elevated by 2.4 fold in the KCN-5 mapping population when compared with other *B. napus* mapping populations. In addition, it was determined that B genome chromosomes can lose terminal loci during F1 meioses, but that surviving B chromosomes are preferentially retained at the BC1 stage of the breeding process.

To determine whether these observations hold true for later generation *B. carinata/B. napus* hybrids, linkage maps and graphical genotypes for the KCN-10 mapping population (BC3) were constructed and will be described in Chapter 5. This will shed light on *B. carinata* introgression occurring at later stages of the backcrossing/introgression program.

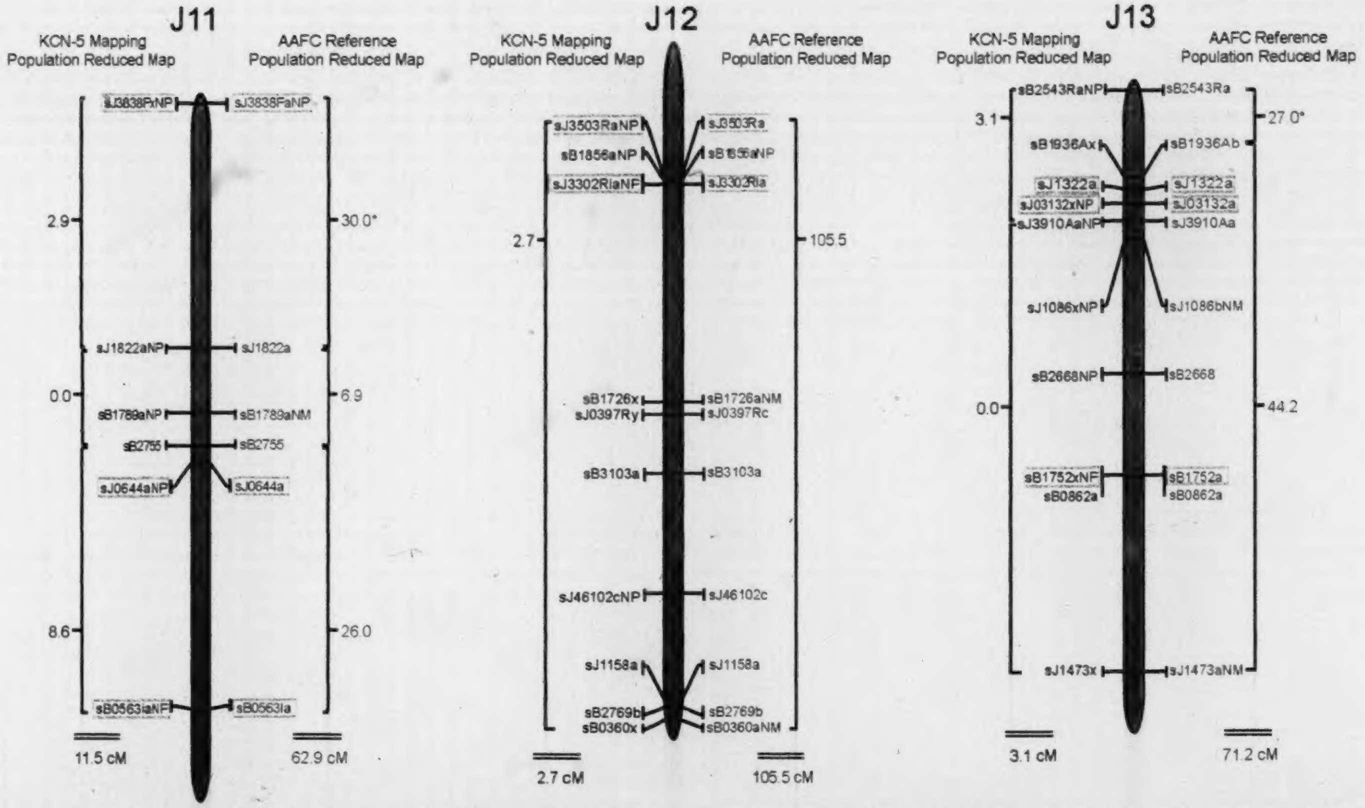

J11
KCN-5 Mapping Population Reduced Map
AAFC Reference Population Reduced Map
sJ3838FaNP
sJ3838FaNP
2.9
30.0*
sJ1822aNP
sJ1822a
0.0
6.9
sB1789aNP
sB1789aNM
sB2755
sB2755
sJ0644aNP
sJ0644a
8.6
26.0
sB0563IaNF
sB0563Ia
11.5 cM
62.9 cM
J12
KCN-5 Mapping Population Reduced Map
AAFC Reference Population Reduced Map
sJ3503RaNP
sJ3503Ra
sB1856aNP
sB1856aNP
sJ3302RIaNF
sJ3302RIa
2.7
105.5
sB1726x
sB1726aNM
sJ0397Ry
sJ0397Rc
sB3103a
sB3103a
sJ46102cNP
sJ46102c
sJ1158a
sJ1158a
sB2769b
sB2769b
sB0360x
sB0360aNM
2.7 cM
105.5 cM
J13
KCN-5 Mapping Population Reduced Map
AAFC Reference Population Reduced Map
sB2543RaNP
sB2543Ra
3.1
27.0*
sB1936Ax
sB1936Ab
sJ1322a
sJ1322a
sJ03132xNP
sJ03132a
sJ3910AaNP
sJ3910Aa
sJ1086xNP
sJ1086bNM
sB2668NP
sB2668
0.0
44.2
sB1752xNF
sB1752a
sB0862a
sB0862a
sJ1473x
sJ1473aNM
3.1 cM
71.2 cM

Figure 4-15: Comparative linkage groups J11, J12 & J13. The graphic contrasts the RF values obtained from the T-1 population, with the pRF values obtained from the KCN-5 mapping population. The RF values from the intraspecific map are to the right of the linkage group, while the *p*RF values obtained from the KCN-5 mapping population are to the left. Markers in grey boxes are publicly available (Lydiate, D and Sharpe, A. available under material transfer agreement). Where an asterisk appears beside the RF, a contributing distance was manually calculated by counting the number of recombination events between the two loci. The total LG RF/*p*RF values for each population are shown at the base of the figure.

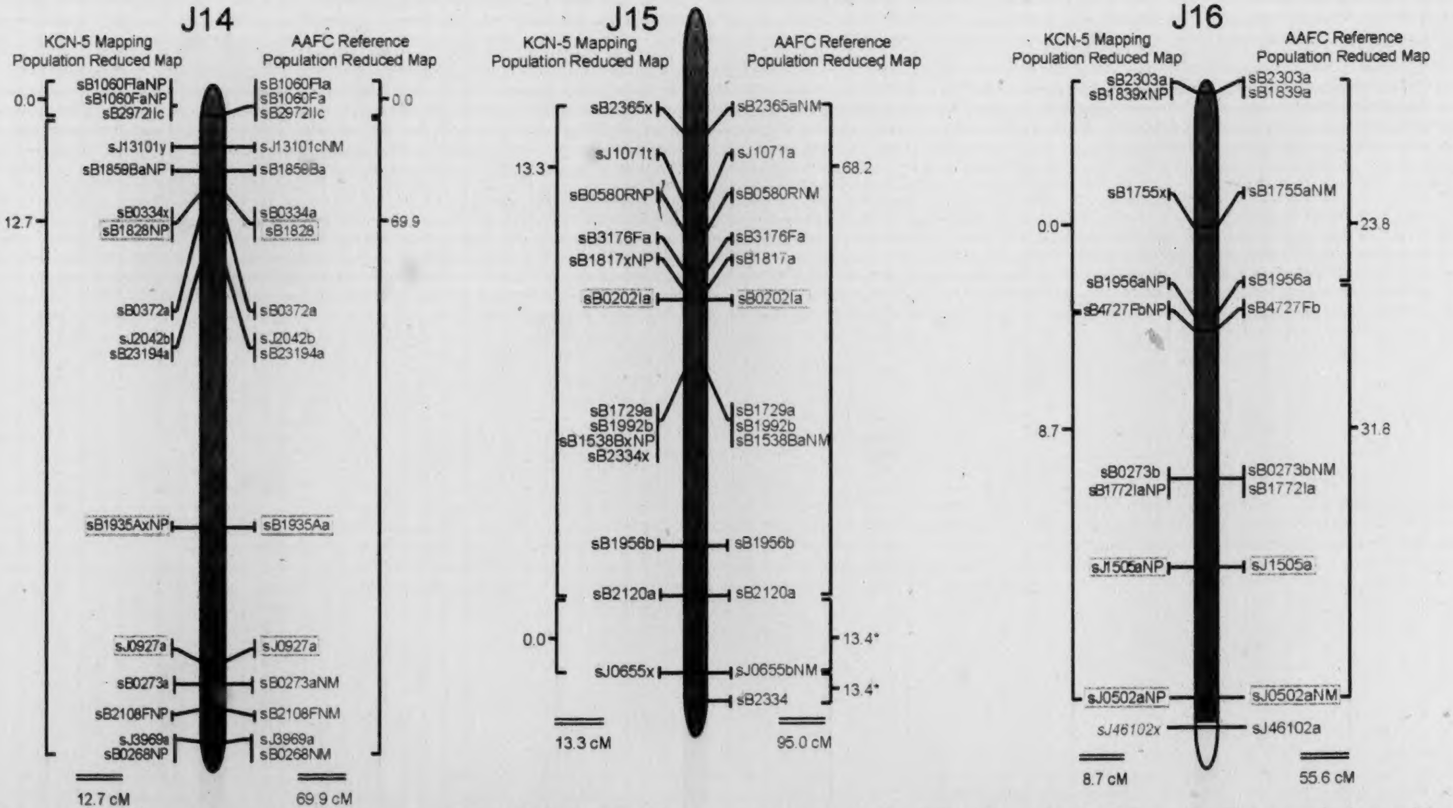

J14
KCN-5 Mapping Population Reduced Map
AAFC Reference Population Reduced Map
0.0
12.7
sB1060FlaNP
sB1060FaNP
sB2972llc
sJ13101y
sB1859BaNP
sB0334x
sB1828NP
sB0372a
sJ2042b
sB23194a
sB1935AxNP
sJ0927a
sB0273a
sB2108FNP
sJ3969a
sB0268NP
sB1060Fla
sB1060Fa
sB2972llc
sJ13101cNM
sB1859Ba
sB0334a
sB1828
sB0372a
sJ2042b
sB23194a
sB1935Aa
sJ0927a
sB0273aNM
sB2108FNM
sJ3969a
sB0268NM
0.0
69.9
12.7 cM
69.9 cM
J15
KCN-5 Mapping Population Reduced Map
AAFC Reference Population Reduced Map
13.3
0.0
sB2365x
sJ1071t
sB0580RNP
sB3176Fa
sB1817xNP
sB0202la
sB1729a
sB1992b
sB1538BxNP
sB2334x
sB1956b
sB2120a
sJ0655x
sB2365aNM
sJ1071a
sB0580RNM
sB3176Fa
sB1817a
sB0202la
sB1729a
sB1992b
sB1538BaNM
sB1956b
sB2120a
sJ0655bNM
sB2334
68.2
13.4*
13.4*
13.3 cM
95.0 cM
J16
KCN-5 Mapping Population Reduced Map
AAFC Reference Population Reduced Map
0.0
8.7
sB2303a
sB1839xNP
sB1755x
sB1956aNP
sB4727FbNP
sB0273b
sB1772laNP
sJ1505aNP
sJ0502aNP
sJ46102x
sB2303a
sB1839a
sB1755aNM
sB1956a
sB4727Fb
sB0273bNM
sB1772la
sJ1505a
sJ0502aNM
sJ46102a
23.8
31.8
8.7 cM
55.6 cM

Figure 4-16: Comparative linkage groups J14, J15 & J16. The graphic contrasts the RF values obtained from the T-1 population, with the pRF values obtained from the KCN-5 mapping population. The RF values from the intraspecific map are to the right of the linkage group, while the *p*RF values obtained from the KCN-5 mapping population are to the left. Markers in grey boxes are publicly available (Lydiate, D and Sharpe, A. available under material transfer agreement). Where an asterisk appears beside the RF, a contributing distance was manually calculated by counting the number of recombination events between the two loci. The total LG RF/*p*RF values for each population are shown at the base of the figure.

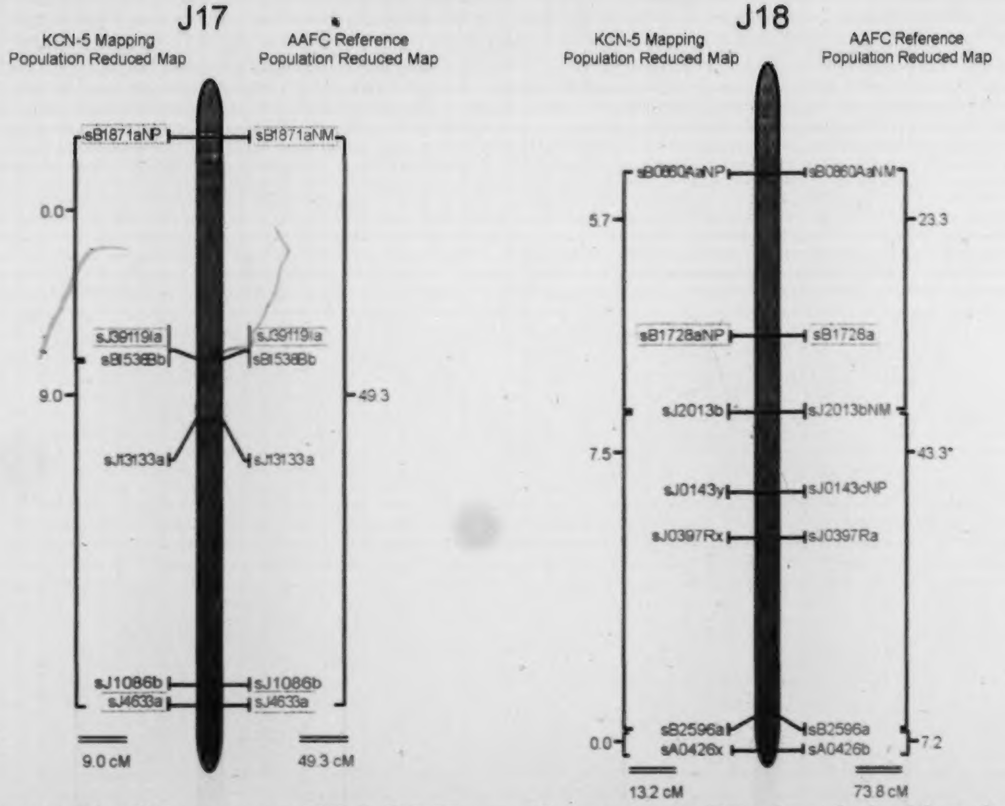

J17
KCN-5 Mapping Population Reduced Map
AAFC Reference Population Reduced Map
sB1871aNP
sB1871aNM
0.0
sJ39119ia
sB1538Bb
sJ39119ia
sB1538Bb
9.0
49.3
sJ13133a
sJ13133a
sJ1086b
sJ4633a
sJ1086b
sJ4633a
9.0 cM
49.3 cM
J18
KCN-5 Mapping Population Reduced Map
AAFC Reference Population Reduced Map
sB0860AaNP
sB0860AaNM
5.7
23.3
sB1728aNP
sB1728a
sJ2013b
sJ2013bNM
7.5
43.3*
sJ0143y
sJ0143cNP
sJ0397Rx
sJ0397Ra
sB2596a
sA0426x
sB2596a
sA0426b
0.0
7.2
13.2 cM
73.8 cM

Figure 4-17: Comparative linkage groups J17 & J18. The graphic contrasts the RF values obtained from the T-1 population, with the pRF values obtained from the KCN-5 mapping population. The RF values from the intraspecific map are to the right of the linkage group, while the *p*RF values obtained from the KCN-5 mapping population are to the left. Markers in grey boxes are publicly available (Lydiate, D and Sharpe, A. available under material transfer agreement). Where an asterisk appears beside the RF, a contributing distance was manually calculated by counting the number of recombination events between the two loci. The total LG RF/*p*RF values for each

2

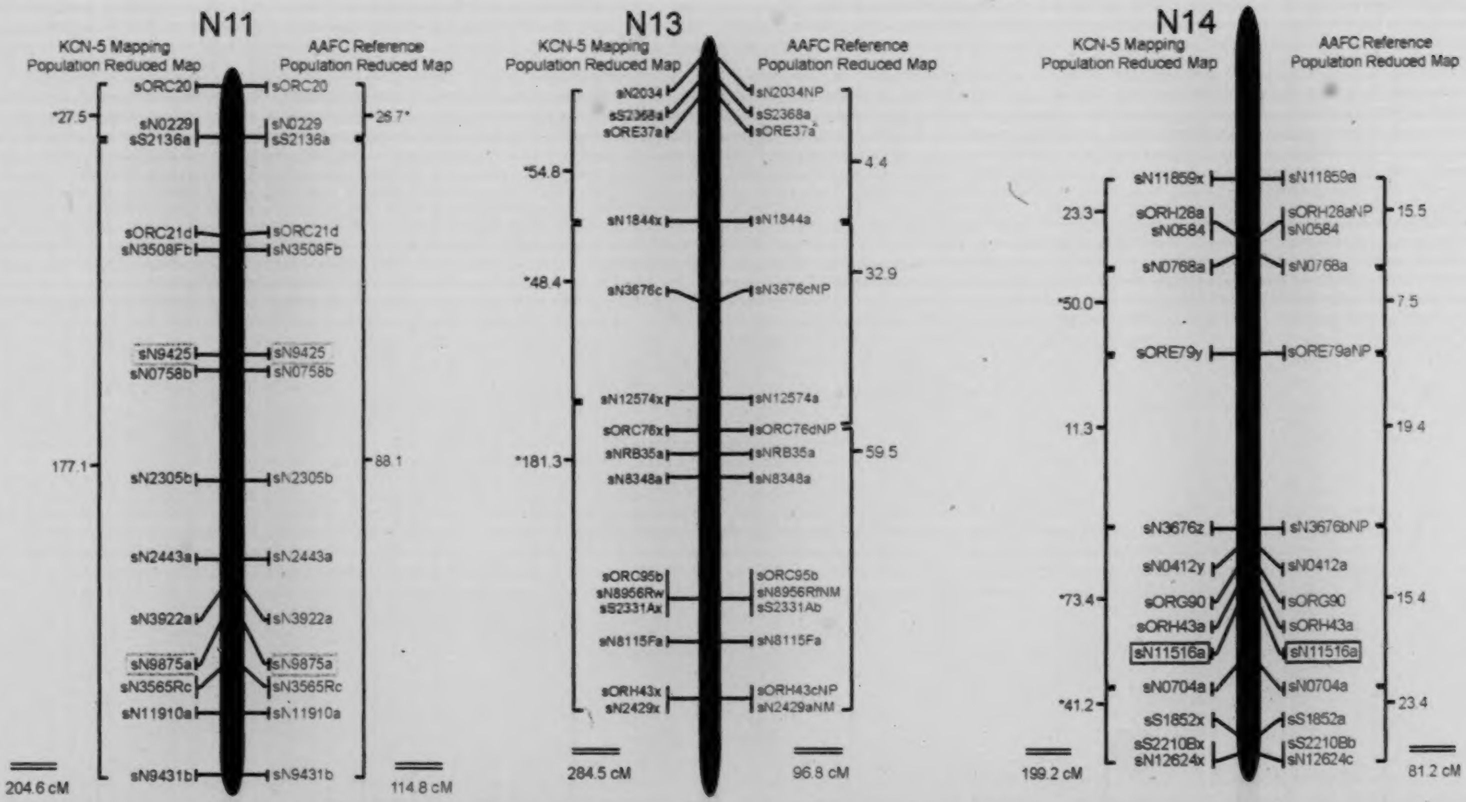

N11
KCN-5 Mapping Population Reduced Map
AAFC Reference Population Reduced Map
sORC20
sN0229
sS2136a
sORC21d
sN3508Fb
sN9425
sN0758b
sN2305b
sN2443a
sN3922a
sN9875a
sN3565Rc
sN11910a
sN9431b
*27.5
177.1
26.7*
88.1
204.6 cM
114.8 cM
N13
KCN-5 Mapping Population Reduced Map
AAFC Reference Population Reduced Map
sN2034
sS2368a
sORE37a
sN2034NP
sN1844x
sN1844a
sN3676c
sN3676cNP
sN12574x
sN12574a
sORC76x
sORC76dNP
sNRB35a
sN8348a
sORC95b
sN8956Rw
sS2331Ax
sN8956RfNM
sS2331Ab
sN8115Fa
sORH43x
sN2429x
sORH43cNP
sN2429aNM
*54.8
*48.4
*181.3
4.4
32.9
59.5
284.5 cM
96.8 cM
N14
KCN-5 Mapping Population Reduced Map
AAFC Reference Population Reduced Map
sN11859x
sN11859a
sORH28a
sN0584
sORH28aNP
sN0768a
sORE79y
sORE79aNP
sN3676z
sN3676bNP
sN0412y
sN0412a
sORG90
sORH43a
sN11516a
sN0704a
sS1852x
sS1852a
sS2210Bx
sN12624x
sS2210Bb
sN12624c
23.3
*50.0
11.3
*73.4
*41.2
15.5
7.5
19.4
15.4
23.4
199.2 cM
81.2 cM

Figure 4-18: Comparative linkage groups N11, N13 & N14. The graphic contrasts the RF values obtained from the 61-9 population, with the RF values obtained from the KCN-5 mapping population. The RF values from the intraspecific map are to the right of the linkage group, while the RF values obtained from the KCN-5 mapping population are to the left. Markers in grey boxes are publicly available (Lydiate, D and Sharpe, A. available under material transfer agreement). Where an asterisk appears beside the RF, a contributing distance was manually calculated by counting the number of recombination events between the two loci. The total LG RF values for each population are shown at the base of the figure.

5. Linkage maps and graphical genotypes for the KCN-10 mapping population

Chapter 4 described the map development of a BC1 population derived from *B. carinata* (BCA-065) x *B. napus* (Topas). This chapter describes a more advanced series of backcrosses, derived from a different set of parents. The choice of MBX as a parent was based on observations by Dr. Lydiate's group that this resynthesized *B. napus* may have reduced meiotic pairing control and therefore higher rates of non homologous recombination between the B genome and the A/C genomes (Parkin *et al.*, 1995). BC1 and BC2 families generated from this cross (KCN-10) were small and therefore unsuitable for use as stand-alone mapping populations. For this reason, the KCN-5 BC1 population was analyzed at the earlier introgression stage. Additionally, using two distinct lineages allowed me to compare two different populations to try and determine if the pattern of inheritance in the KCN-5 population was a general phenomenon, or specific to the lineage. Primarily, the KCN-10 BC3 mapping population provided information on introgression occurring to the BC3 stage of the breeding process.

Chapter 4 discussed the selection and evaluation of SSR markers that were used to align linkage groups and construct maps for the linkage groups of the KCN-5 mapping population, and the KCN-5 BC2 Sibling family. The same set of criteria was employed to evaluate the markers used to characterize the KCN-10 mapping population, as well as the KCN-10 BC1 and BC2 Sibling families.

5.1. Selecting SSR markers for the evaluation of KCN-10 germplasm

Molecular markers that were used to characterize the KCN-10 germplasm were selected and evaluated as described in Chapter 4. The progenitors of the KCN-10 mapping population were included on the screening gel panels (SGs) (Figure 4-3) and the Sibling assay panels (Figure 4-6). The 220 SSR marker subset described in Chapter 4 was used to assay the Sibling families. The additional screens were necessary as it was a challenge to find *B. carinata* alleles in the BC3 germplasm. This was especially true of C genome specific markers, as the backcrossing process leads to the inheritance of primarily monomorphic *B. napus* alleles.

In addition to the AAFC reference map alleles and the alleles found in the representative *Brassica* genomes, the KCN-5 mapping population allele sizes were used as a reference for assigning markers to a linkage group in the KCN-10 germplasm (Parkin and Lydiate 1997; Axellson *et al.*, 2000). For example, if a locus did not correspond to any allele sizes in the AAFC reference maps, but the allele was mapped to a linkage group in the KCN-5 mapping population, it was allocated to the same LG in the KCN-10 germplasm (Figure 4-3, A and B). In cases where KCN-5 information was used, all B genome alleles were properly designated using this method, and there was no evidence that any C genome allele was incorrectly assigned. Generally, loci matched allele sizes in at least two reference maps, these alleles were then used to construct and orient the KCN-10 population linkage groups.

5.2. Assembly of linkage groups comprising the C and B genomes of the KCN-10 mapping population

The B and C genome linkage groups of the KCN-10 mapping population were designated by aligning putative linkage groups with the AAFC reference maps via common marker alleles (Parkin and Lydiate 1997; Axellson *et al.*, 2000). KCN-10-2-11 carried loci from only five B genome linkage groups; hence markers amplifying alleles associated with these five LGs (J13, J15, J16, J17 and J18) were analyzed on mapping panels. Nevertheless, a selection of markers known to amplify evenly spaced loci, including terminal loci, on J11, J12 and J14 were also included on the KCN-10 mapping population SSR panels. From this set of markers, 19 alleles specific to J11, J12 and J14 were confirmed to be absent from the mapping population (Appendix A). Most J15 alleles were also found to be absent using this method (Section 5.6). The C genome alleles from *B. carinata* were also aligned using AAFC reference maps, and KCN-5 mapping population. Most of the loci from the *B. carinata* C genome were not conserved in the BC3 population (due to backcrossing to *B. napus*), therefore the KCN-10 Sibling families allowed for the identification of *B. carinata* allele loss from the F1 generation to the BC3 generation.

The KCN-10 mapping population scoring matrix data generated a linkage map of the B and C genomes consisting of 12 identified LGs/segments, 78 mapped loci and eight unassigned loci. These figures did not include the large group of informative alleles that were lost during the backcross process, and therefore were not present in the KCN-10 mapping population.

The experimental germplasm was initially screened with the 406 primer pairs discussed in Chapter 4. The majority of informative SSR markers were not present in KCN-10-2-11; therefore, characterized marker loci were assayed in the Sibling families to determine the generation at which they were excluded. For the KCN-10 germplasm, 142 alleles were associated with the B genome LGs and 119 alleles were associated with the C genome LGs (261 total). Of these, 63 B genome alleles (44%) and 19 C genome alleles (16%) were retained in the KCN-10 mapping population (Table 5-1).

The RF/*p*RF values derived from the KCN-10 mapping population are shown in Table 5-1. It should be noted that the RF values derived from C genome data were the result of recombination, but the *p*RF (*pseudo*-RF) values derived from the B genome scoring matrix were the result of loci loss, and not recombination. The B genome LGs J13, J17 and J18 were missing small terminal segments not accounted for in the *p*RF values, while J16 was conserved. The longest measured span was 13.9 cM, down to 2.3 cM in J13. RF values for portions of the mapping population C genome LGs could be calculated, and are shown in Table 5-1. Segmentation of the C genome made it unfeasible to construct a robust linkage map of the C genome LGs, as only small introgressed spans were evident. Visual representations of the excluded C genome alleles can be seen in Figures 5-7 and 5-8. Although the number of C genome alleles conserved in the BC1 and BC2 Sibling families was much higher, these families were too small to derive statistically sound RF values from their scoring matrices (Table 5-1, and Figures 5-1 to 5-8).

5.3. Comparative recombination frequencies of the C and B interspecific genomes

When compared to the AAFC reference maps, the C genome of the KCN-10 mapping population showed inflated RF values, while the B genome *p*RF values were depressed (Table 5-1). For the C genome, seven linked spans were derived from the scoring matrices (Appendix A). These spans corresponded to the loci that were present in the progenitor plant (KCN-10-2-11). The two loci span on N11 (Figure 5-7) was 13.7 cM, and the N13 mapped span between sR6212y and sN1838y was 11.5 cM. The manually calculated distance from this span to sB2972iix was 37.5 cM (Figure 5-8). The comparable N11 AAFC reference map distance was 13.7 cM (Figure 4-18). A comparison between the comparable AAFC reference map N13 span was not applicable as only one of the four alleles was found in the AAFC reference maps. N17, which had the most conserved loci from *B. carinata* (Table 5-1), had a RF between the mapped alleles of 33.8 cM, while the AAFC reference map value for the same span was 10.2 cM. Hence, both C genome LGs spans exhibited just over double (2.25 fold) the RF that would be expected according to the AAFC reference maps. The remaining segments did not have enough AAFC corresponding alleles to make a comparison.

Conversely, the loci corresponding to the B genome appeared tightly 'linked'. Like the KCN-5 mapping population, most of the B genome alleles mapped coincidentally with other alleles on the same linkage group. The inherited portion of J13 spanned just 2.3 cM, where the AAFC map exhibited 44.2 cM for a subset of the same loci (Figure 4-15). J16 spanned 5.8 cM, but a subset of shared AAFC loci exhibited a J16 *p*RF of 60.6 cM (Figure 4-16). Likewise, the KCN-10 BC3 partial J17 segment was 3.7 cM and the partial J18 segment spanned 13.9 cM, but the corresponding AAFC segments were 49.3 cM and 66.6 cM respectively (Figure 4-17). Thus, the AAFC RF values for the B genome LGs were (at least) an average of ten-fold higher than the KCN-10 mapping population *p*RF values. The J15 segment will be discussed in section 5.6.

Table 5-1: Allele loss and RF/*p*RF values associated with the KCN-10 mapping population. The table indicates the number of evenly distributed alleles that were present in the F1 KCN-10 and the corresponding alleles remaining in the BC3 generation. The RF/*p*RF values associated with each LG or LG segment are also shown. RF values marked with an asterisk were small *B. carinata* segments introgressed into the *B. napus* genome. The *p*RF values from J13, J16, J17 and J18 were full, or near full length chromosomes. If "n/a" (not applicable) is present instead of a RF value, the LG was either absent or, in the case of C genome LGs, not enough loci were present to derive a RF. Where the AAFC RF values are shown; 'n/a' means that there were not enough alleles in common between the populations to make a comparison. RF values denoted by a double asterisk indicate that the measurement represents a subset of AAFC loci. Hence, there were often more loci on the KCN-10 mapping population linkage map. Thus the actual difference between the two populations could be underestimated.

B Genome LG	F1 alleles present	KCN-10 BC3 alleles	pRF(CM)	RF (cM) AAFC reference map Subset	C Genome LG	F1 alleles present	KCN-10 BC3 alleles	RF(CM)	RF (cM) AAFC reference map Subset
J11	15	0	n/a	n/a	N11	15	2	13.7*	13.7
J12	16	0	n/a	n/a	N12	13	2	27.5*	n/a
J13	19	14	2.3	44.2**	N13	23	4	49.0*	n/a
J14	21	0	n/a	n/a	N14	18	1	n/a	n/a
J15	22	3	2.8*	0.0**	N15	9	0	n/a	n/a
J16	17	17	5.8	60.6**	N16	6	0	n/a	n/a
J17	16	14	3.7	49.5**	N17	14	5	33.8*	10.2
J18	16	15	13.9	66.6**	N18	10	2	34.3*	n/a
Total	142	63			N19	11	3	20.7*	unlinked
					Total	119	19		

5.4. Graphical genotypes for the KCN-10 mapping population and Sibling families

Through the combination of BC3 RF/*p*RF values and allele identities established by reference maps (AAFC maps and KCN-5 mapping population), it was possible to build graphical diagrams of the progressive loss or retention of loci through the backcrossing process. The resulting graphical genotypes illustrate the probable locations of chromosome disruptions (B genome) or recombination (C genome). Figures 5-1 to 5-6 are the B genome graphical genotypes of the KCN-10 BC1 and BC2 Sibling families, as well as the KCN-10 mapping population. Figures 5-7 and 5-8 characterize the same germplasm and represent the C genome LGs N11 and N13. Scoring matrix data was gathered for the loci associated with the remaining C genome LGs (N12, N14-N19), and confirmed the trends observed for N11 and N13 (Appendix A). When interpreting Figures 5-1 to 5-8, the ideograms show the alleles recognized in the F1 KCN-10 and represent the recombination events/allele loss that was evident in a lineage of BC1, BC2 and BC3 plants derived from it. The graphical genotypes are not drawn to scale; the SSR markers (on the left of ideograms) are evenly distributed along the LG. Horizontal lines drawn from some loci highlight SSR markers that also amplified alleles on this LG in the AAFC reference maps. Markers without extended horizontal lines were known to amplify alleles on other LGs in the AAFC reference maps, but were assigned to this LG as new alleles. The yellow shaded tags highlight the maternal parents to the subsequent family. Shaded sections of the ideogram represent the presence of *B. carinata* alleles, while the white areas indicate the absence of *B. carinata* alleles. Hatched sections indicate regions between markers where a LG disruption or a recombination event occurred. Where a hatched section spans a locus, that locus was missing a data point at that location (failed PCR reaction). Hence, a recombination event/allele loss could have occurred, but could not be monitored (Figures 5-1 to 5-8, at end of chapter).

5.5. Segregation distortion in the KCN-10 mapping population

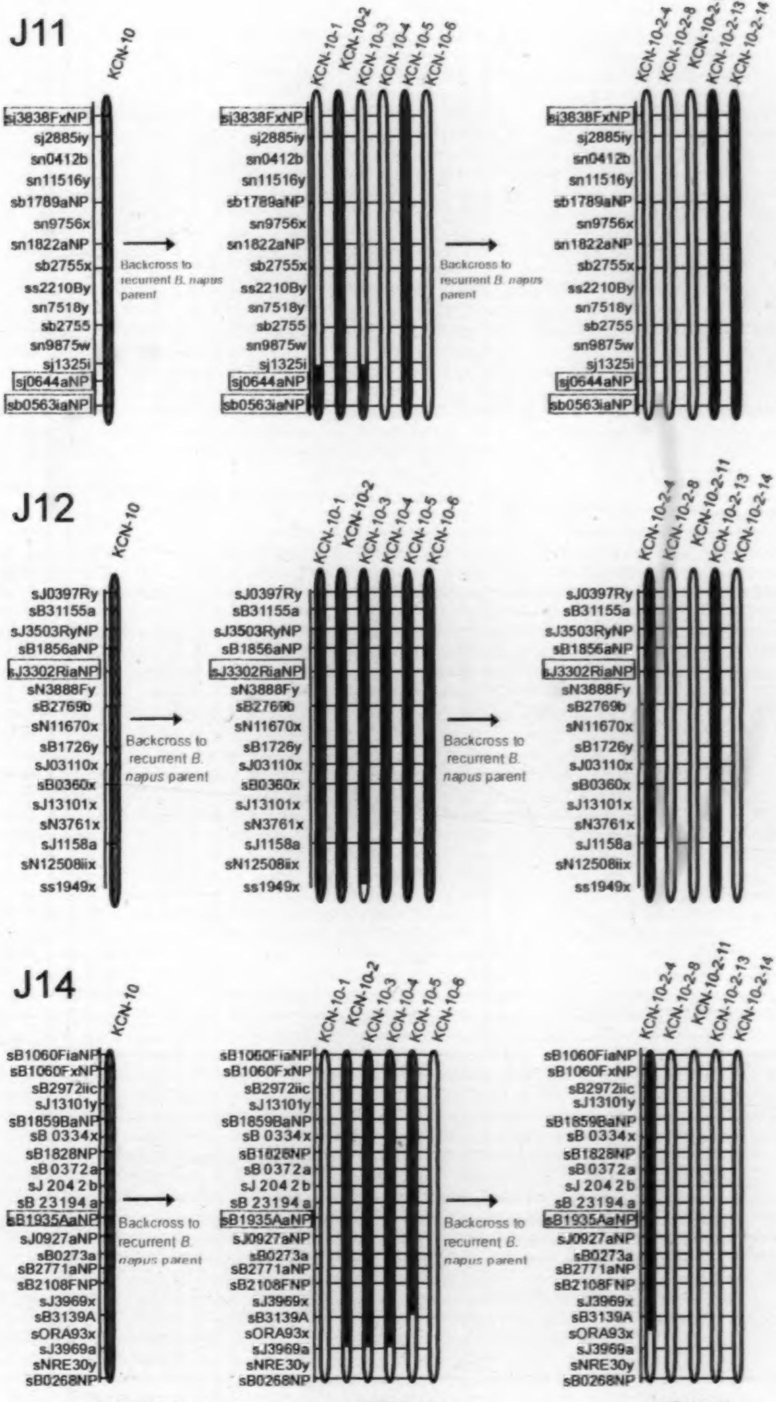
The observed rate of conserved *B. carinata* C genome alleles in the BC3 generation was 16% in the BC3 population, while 44% of the *B. carinata* B genome loci were conserved. However, these values were skewed by the inheritance of entire B genome chromosomes instead of small introgressed segments (Figures 5-1 to 5-6). If the chromosome inheritance was observed for each BC3 B genome chromosome, with even a smaller segment deemed a whole chromosome, the frequency of a B genome chromosome being passed on to the BC3 generation ranged from 20% (J17 and J18, Figures 5-5 and 5-6) to 33% (J13, Figure 5-2). These inherited B genome chromosome values were below the 1:1 ratio that was observed in previous generations.

In terms of the segregation distortion of the BC3 loci, it would be expected that loci that were present in KCN-10-2-11 would be inherited like a normal Mendelian trait (50% frequency) in the next generation. It was observed that the B genome loci tended to be inherited at a much lower rate in the KCN-10 mapping population. Marker scorings for each locus in the B and C genomes were tested for segregation distortion using a chi-squared (x^2) statistical test, and an expected allele ratio of 1:1; excluding any missing data points. The loci of the B genome were highly distorted; showing a significant reduction in the number of B genome loci being inherited in the next generation (Table 5-2). The alleles, sJ1071b, sB0273b and sJ1505a were exceptions; however this result was attributed to the position of missing data points in the scorings. Loci attributed to J15 were also exceptions and were inherited in a 1:1 ratio. These results are discussed in Section 5.6.

A normal Mendelian segregation frequency was observed for the alleles associated with the C genome (Table 5-3). All of the C genome loci associated with mapping data did not show a deviation from the $H_{0\,(1:1)}$. Also, the unassociated loci in the scoring data all inherited their *B. carinata* allele in a 1:1 ratio, suggesting that they were probably unassigned C genome alleles that could not be linked to a LG due to inflated RF values in the cross, and a low number of small inherited C genome segments in the advanced backcross. The difference in inheritance ratios between the alleles of each genome were so distinct that alleles could be designated as being C or B genome based largely on segregation ratios.

When looking at the smaller progenitor families of the KCN-10 mapping population, it was apparent that certain B genome chromosomes were preferentially inherited. Table 5-4 is an overview of the inherited B genome chromosomes for the KCN-10 Sibling families. All B genome chromosomes were present in the F1 KCN-10, and most of the B chromosomes were maintained in the BC1 individuals with an average conservation of 82%. The percentage of retained B genome/KCN-10-2 chromosomes fell to 54% in the BC2 generation (Table 5-4; Figure 5-9). The KCN-10 BC1 family showed slightly higher B genome retention than the KCN-5 mapping population, and similar values to the BC2 KCN-5 family. Comparisons between the two lineages (KCN-5 and KCN-10) were not significant as the sibling families contained too few representatives.

Figure 5-1: Graphical genotypes of J11, J12 & J14 for the KCN-10 Sibling families. The KCN-10 mapping population is not included as J11, J12 and J14 chromosomes were not inherited by KCN-10-2-11. This was verified by running anchoring markers in the mapping population. Lightly shaded tags indicate the donor parent for the next generation. Labels to the left of the ideograms are SSR markers that were assigned to the linkage group through allele identities on the KCN-5 mapping population and the T-1 reference population. Markers in the grey boxes are publicly available (Lydiate, D. and Sharpe, A., available through material transfer agreement). Shaded portions of the linkage group indicate the presence of *B.carinata* alleles; un-shaded areas indicate the absence of *B.carinata* alleles at the specified loci. The hatched intervals indicate junctions where chromosome disruption occurred, leading to a loss of donor loci beyond the hatched region.


J11
KCN-10
KCN-10-1
KCN-10-2
KCN-10-3
KCN-10-4
KCN-10-5
KCN-10-6
KCN-10-2-4
KCN-10-2-8
KCN-10-2-11
KCN-10-2-13
KCN-10-2-14
sj3838FxNP
sj2885iy
sn0412b
sn11516y
sb1789aNP
sn9756x
sn1822aNP
sb2755x
ss2210By
sn7518y
sb2755
sn9875w
sj1325i
sj0644aNP
sb0563iaNP
Backcross to recurrent B. napus parent
J12
sJ0397Ry
sB31155a
sJ3503RyNP
sB1856aNP
sJ3302RiaNP
sN3888Fy
sB2769b
sN11670x
sB1726y
sJ03110x
sB0360x
sJ13101x
sN3761x
sJ1158a
sN12508iix
ss1949x
J14
sB1060FiaNP
sB1060FxNP
sB2972iic
sJ13101y
sB1859BaNP
sB 0334x
sB1828NP
sB 0372a
sJ 204 2b
sB 23194 a
sB1935AaNP
sJ0927aNP
sB0273a
sB2771aNP
sB2108FNP
sJ3969x
sB3139A
sORA93x
sJ3969a
sNRE30y
sB0268NP
F1 KCN-10
KCN-10
BC1 Family
KCN-10
BC2 Family

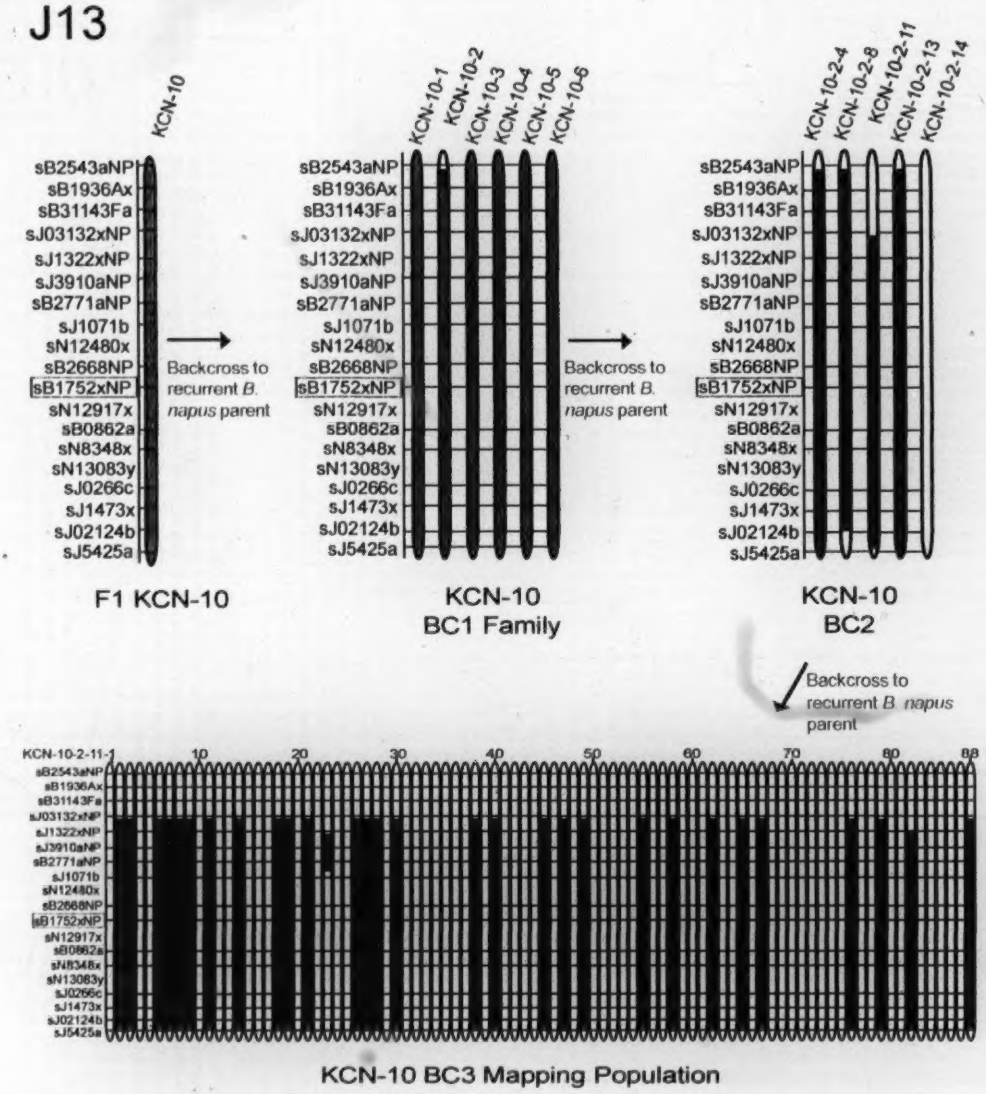


Figure 5-2: Graphical genotypes of J13 for the KCN-10 Sibling families and KCN-10 Mapping Population. Lightly shaded tags indicate the donor parent for the next generation. Labels to the left of the ideograms are SSR markers that were assigned to the linkage group through allele identities on the KCN-5 mapping population and the T-1 reference population. Markers in the grey boxes are publicly available (Lydiate, D. and Sharpe, A., available through material transfer agreement). Shaded portions of the linkage group indicate the presence of *B.carinata* alleles, unshaded areas indicate the absence of *B.carinata* alleles at the specified loci. The hatched intervals indicate junctions where chromosome disruption ocurred leading to a loss of donor loci beyond the hatched region.

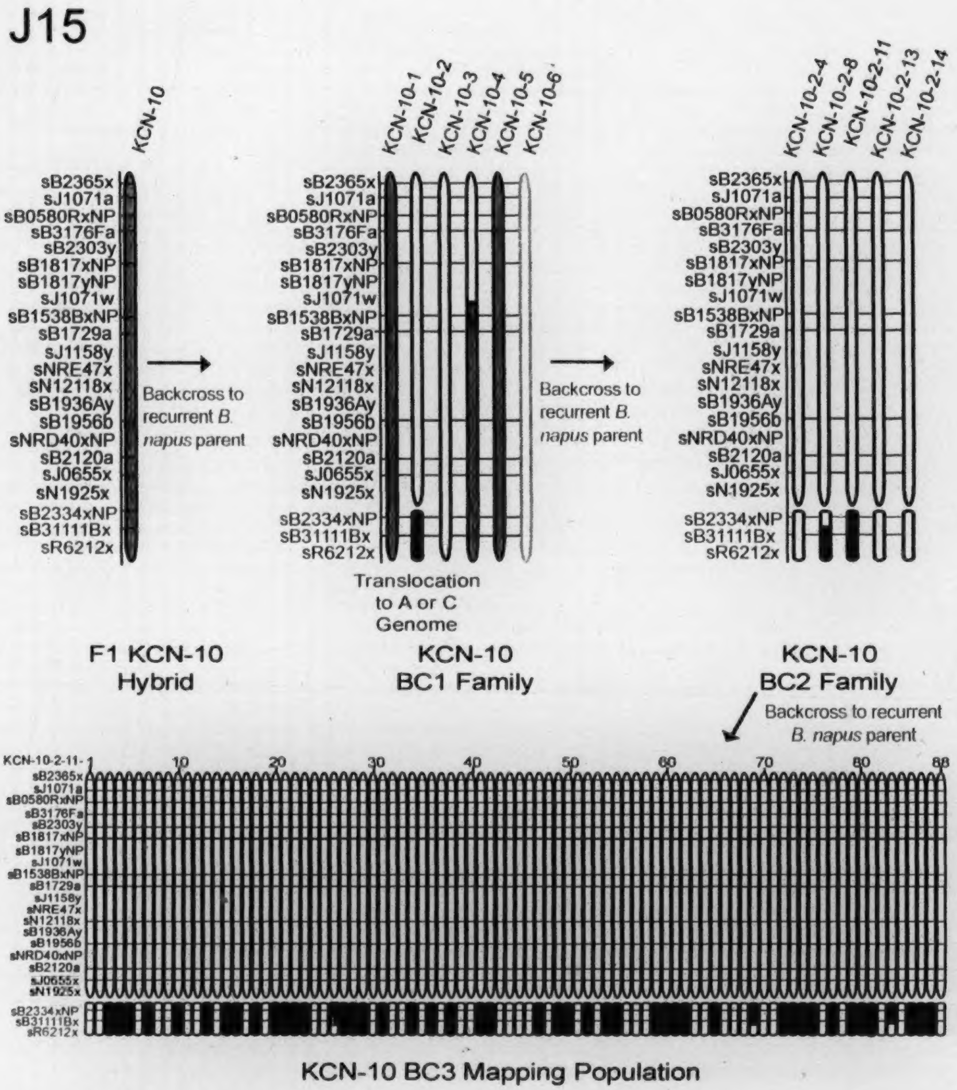

J15
KCN-10
sB2365x
sJ1071a
sB0580RxNP
sB3176Fa
sB2303y
sB1817xNP
sB1817yNP
sJ1071w
sB1538BxNP
sB1729a
sJ1158y
sNRE47x
sN12118x
sB1936Ay
sB1956b
sNRD40xNP
sB2120a
sJ0655x
sN1925x
sB2334xNP
sB31111Bx
sR6212x
Backcross to recurrent B. napus parent
KCN-10-1
KCN-10-2
KCN-10-3
KCN-10-4
KCN-10-5
KCN-10-6
Translocation to A or C Genome
Backcross to recurrent B. napus parent
KCN-10-2-4
KCN-10-2-8
KCN-10-2-11
KCN-10-2-13
KCN-10-2-14
F1 KCN-10 Hybrid
KCN-10 BC1 Family
KCN-10 BC2 Family
Backcross to recurrent B. napus parent
KCN-10-2-11- 1
10
20
30
40
50
60
70
80
88
KCN-10 BC3 Mapping Population

Figure 5-3: Graphical genotypes of J15 for the KCN-10 Sibling families and KCN-10 Mapping Population. Lightly shaded tags indicate the donor parent for the next generation. Labels to the left of the ideograms are SSR markers that were assigned to the linkage group through allele identities on the KCN-5 mapping population and the T-1 reference population. Markers in the grey boxes are publicly available (Lydiate, D. and Sharpe, A., available through material transfer agreement). Shaded portions of the linkage group indicate the presence of *B.carinata* alleles, unshaded areas indicate the absence of *B.carinata* alleles at the specified loci. The hatched intervals indicate junctions where chromosome disruption ocurred leading to a loss of donor loci beyond the hatched region. The darkest segments are alleles that have been introgressed into the C or A genome and are unlinked to J15. Data was not available for KCN-10-6.

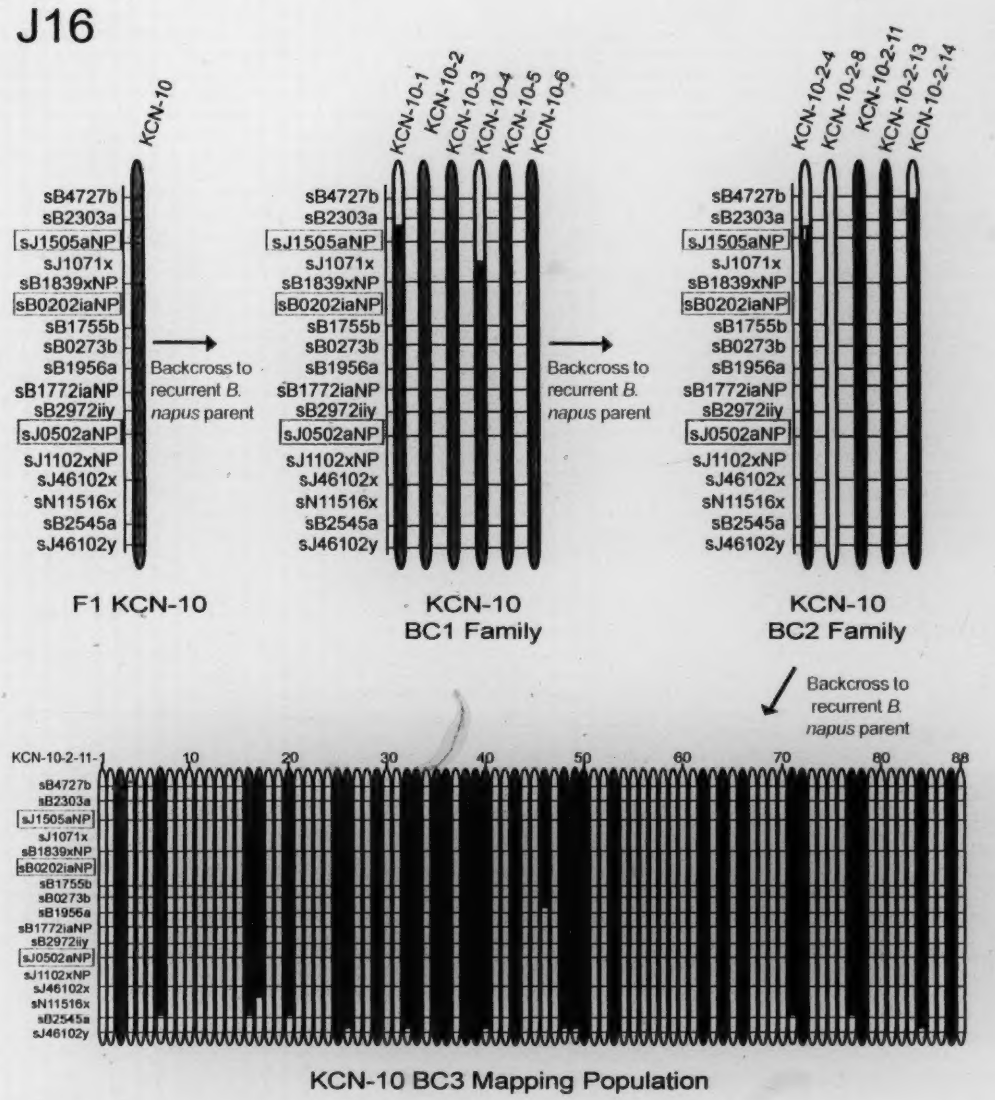

J16
KCN-10
KCN-10-1
KCN-10-2
KCN-10-3
KCN-10-4
KCN-10-5
KCN-10-6
KCN-10-2-4
KCN-10-2-8
KCN-10-2-11
KCN-10-2-13
KCN-10-2-14
sB4727b
sB2303a
sJ1505aNP
sJ1071x
sB1839xNP
sB0202iaNP
sB1755b
sB0273b
sB1956a
sB1772iaNP
sB2972iiy
sJ0502aNP
sJ1102xNP
sJ46102x
sN11516x
sB2545a
sJ46102y
Backcross to recurrent *B. napus* parent
Backcross to recurrent *B. napus* parent
Backcross to recurrent *B. napus* parent
F1 KCN-10
KCN-10
BC1 Family
KCN-10
BC2 Family
KCN-10-2-11-1
10
20
30
40
50
60
70
80
88
KCN-10 BC3 Mapping Population

Figure 5-4: Graphical genotypes of J16 for the KCN-10 Sibling families and KCN-10 Mapping Population. Lightly shaded tags indicate the donor parent for the next generation. Labels to the left of the ideograms are SSR markers that were assigned to the linkage group through allele identities on the KCN-5 mapping population and the T-1 reference population. Markers in the grey boxes are publicly available (Lydiate, D. and Sharpe, A., available through material transfer agreement). Shaded portions of the linkage group indicate the presence of *B.carinata* alleles, unshaded areas indicate the absence of *B.carinata* alleles at the specified loci. The hatched intervals indicate junctions where chromosome disruption ocurred leading to a loss of donor loci beyond the hatched region.

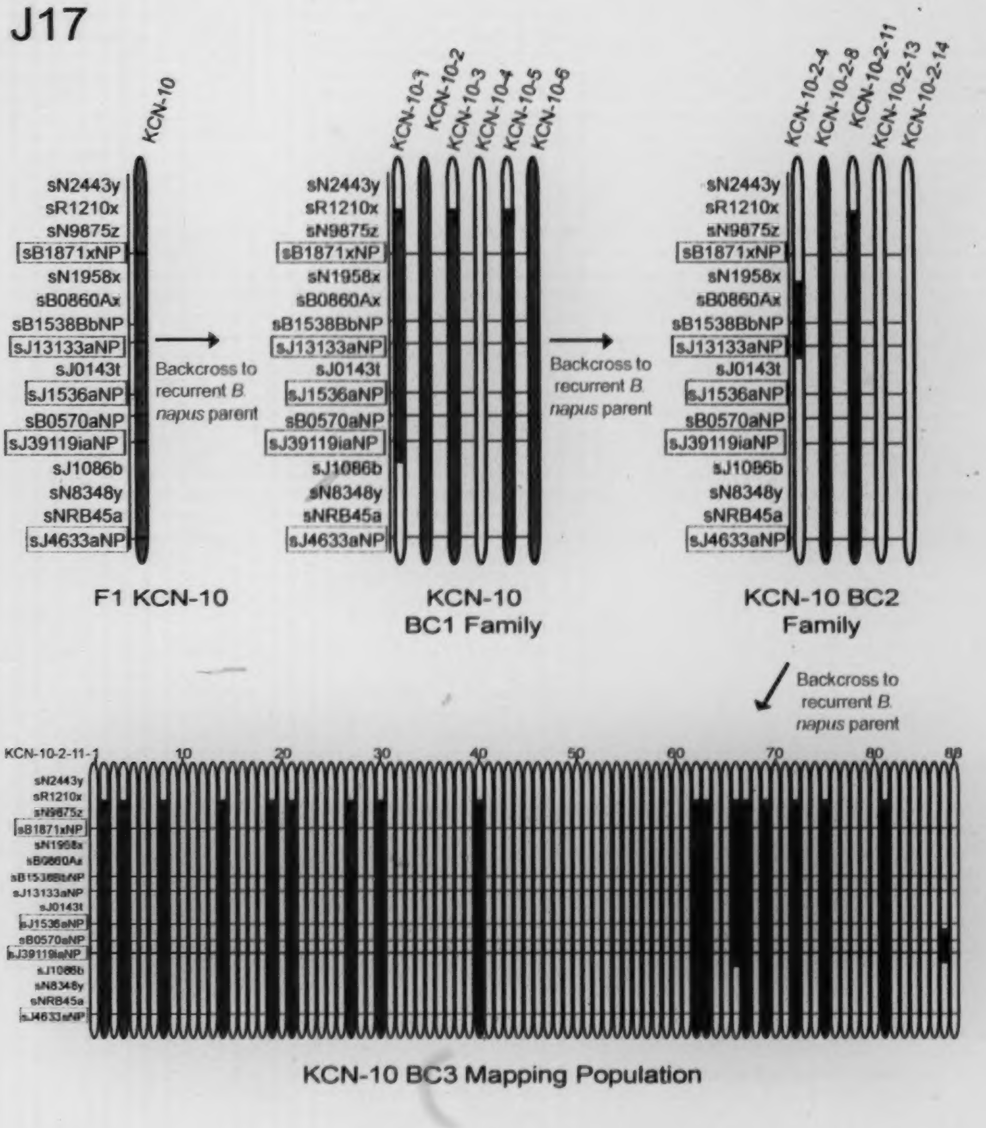

J17
KCN-10
sN2443y
sR1210x
sN9875z
sB1871xNP
sN1958x
sB0860Ax
sB1538BbNP
sJ13133aNP
sJ0143t
sJ1536aNP
sB0570aNP
sJ39119iaNP
sJ1086b
sN8348y
sNRB45a
sJ4633aNP
Backcross to recurrent B. napus parent
F1 KCN-10
KCN-10-1
KCN-10-2
KCN-10-3
KCN-10-4
KCN-10-5
KCN-10-6
KCN-10 BC1 Family
KCN-10-2-4
KCN-10-2-8
KCN-10-2-11
KCN-10-2-13
KCN-10-2-14
KCN-10 BC2 Family
KCN-10-2-11-1
10
20
30
40
50
60
70
80
88
KCN-10 BC3 Mapping Population

Figure 5-5: Graphical genotypes of J17 for the KCN-10 Sibling families and KCN-10 Mapping Population. Lightly shaded tags indicate the donor parent for the next generation. Labels to the left of the ideograms are SSR markers that were assigned to the linkage group through allele identities on the KCN-5 mapping population and the T-1 reference population. Markers in the grey boxes are publicly available (Lydiate, D. and Sharpe, A., available through material transfer agreement). Shaded portions of the linkage group indicate the presence of *B.carinata* alleles, unshaded areas indicate the absence of *B.carinata* alleles at the specified loci. The hatched intervals indicate junctions where chromosome disruption ocurred leading to a loss of donor loci beyond the hatched region.

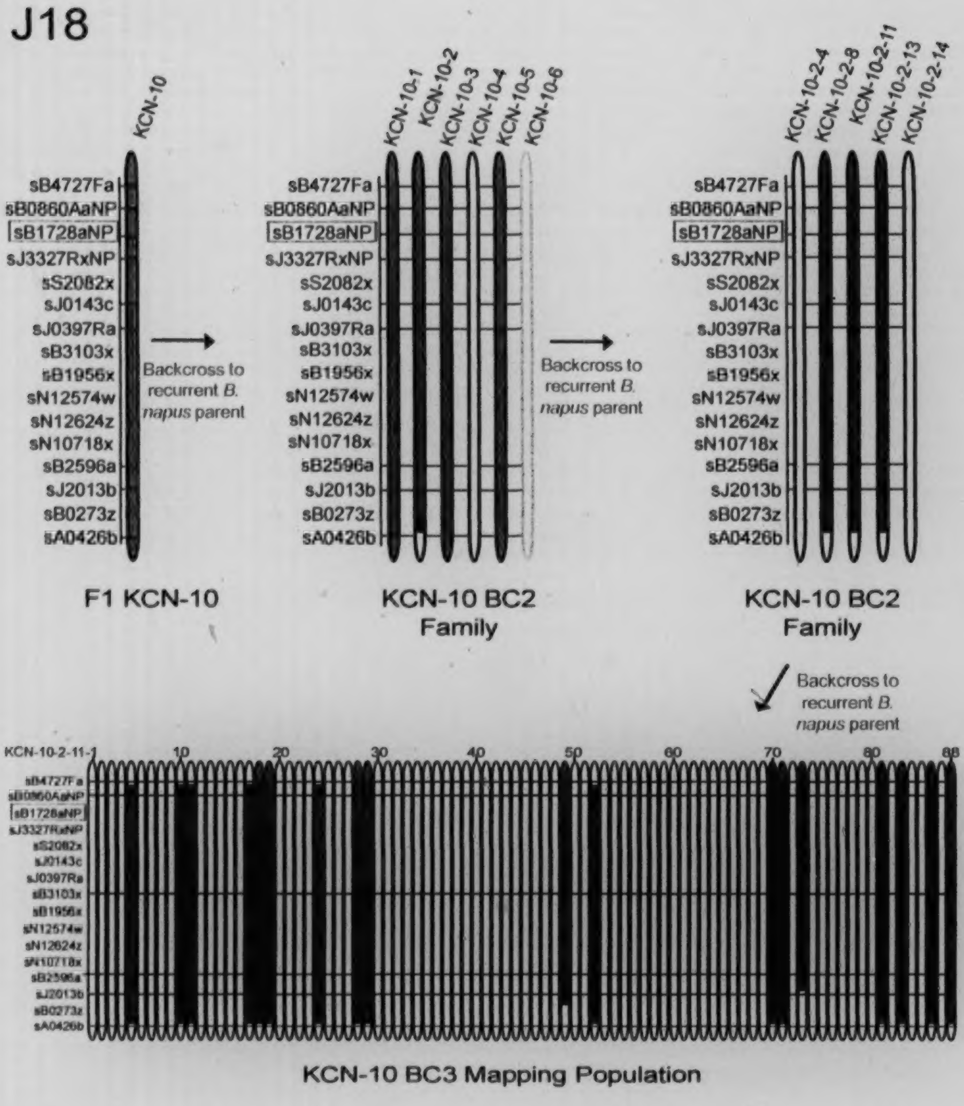

J18
KCN-10
sB4727Fa
sB0860AaNP
sB1728aNP
sJ3327RxNP
sS2082x
sJ0143c
sJ0397Ra
sB3103x
sB1956x
sN12574w
sN12624z
sN10718x
sB2596a
sJ2013b
sB0273z
sA0426b
Backcross to recurrent B. napus parent
F1 KCN-10
KCN-10-1
KCN-10-2
KCN-10-3
KCN-10-4
KCN-10-5
KCN-10-6
KCN-10 BC2 Family
KCN-10-2-4
KCN-10-2-8
KCN-10-2-11
KCN-10-2-13
KCN-10-2-14
KCN-10 BC2 Family
Backcross to recurrent B. napus parent
KCN-10-2-11-1
10
20
30
40
50
60
70
80
88
KCN-10 BC3 Mapping Population

Figure 5-6: Graphical genotypes of J18 for the KCN-10 Sibling families and KCN-10 Mapping Population. Lightly shaded tags indicate the donor parent for the next generation. Labels to the left of the ideograms are SSR markers that were assigned to the linkage group through allele identities on the KCN-5 mapping population and the T-1 reference population. Markers in the grey boxes are publicly available (Lydiate, D. and Sharpe, A., available through material transfer agreement). Shaded portions of the linkage group indicate the presence of *B.carinata* alleles, unshaded areas indicate the absence of *B.carinata* alleles at the specified loci. The hatched intervals indicate junctions where chromosome disruption ocurred leading to a loss of donor loci beyond the hatched region. Data was unavailable for KCN-10-2-6.

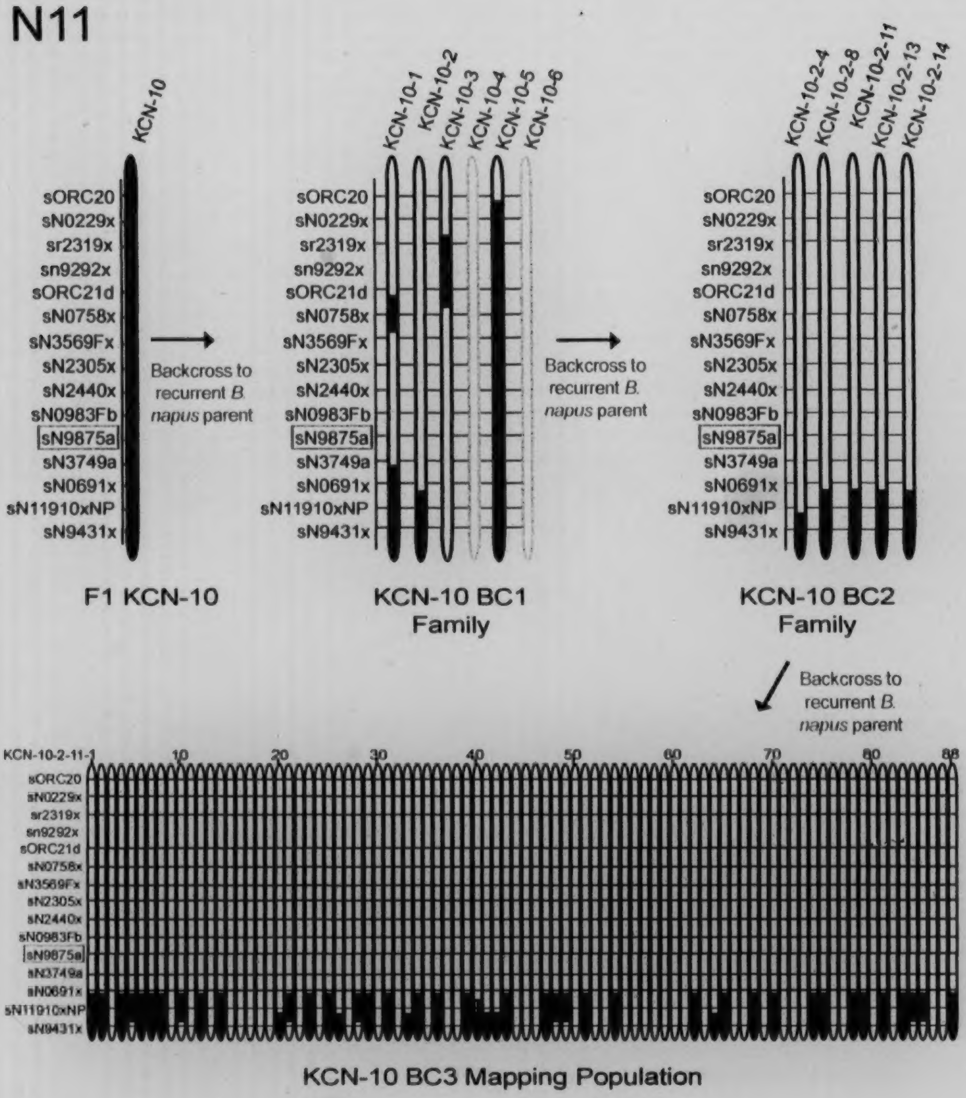

N11
KCN-10
sORC20
sN0229x
sr2319x
sn9292x
sORC21d
sN0758x
sN3569Fx
sN2305x
sN2440x
sN0983Fb
sN9875a
sN3749a
sN0691x
sN11910xNP
sN9431x
Backcross to recurrent B. napus parent
F1 KCN-10
KCN-10-1
KCN-10-2
KCN-10-3
KCN-10-4
KCN-10-5
KCN-10-6
KCN-10 BC1 Family
KCN-10-2-4
KCN-10-2-8
KCN-10-2-11
KCN-10-2-13
KCN-10-2-14
KCN-10 BC2 Family
KCN-10-2-11-1
10
20
30
40
50
60
70
80
88
KCN-10 BC3 Mapping Population

Figure 5-7: Graphical genotypes of N11 for the KCN-10 Sibling families and KCN-10 Mapping Population. Lightly shaded tags indicate the donor parent for the next generation. Labels to the left of the ideograms are SSR markers that were assigned to the linkage group through allele identities on the KCN-5 mapping population and the 61-9 reference population. Markers in the grey boxes are publicly available (Lydiate, D. and Sharpe, A., available through material transfer agreement). Shaded portions of the linkage group indicate the presence of *B.carinata* alleles, unshaded areas indicate the absence of *B.carinata* alleles at the specified loci. The hatched intervals indicate junctions where chromosome disruption ocurred leading to a loss of donor loci beyond the hatched region. Data was unavailable for KCN-10-2-4 and -6.

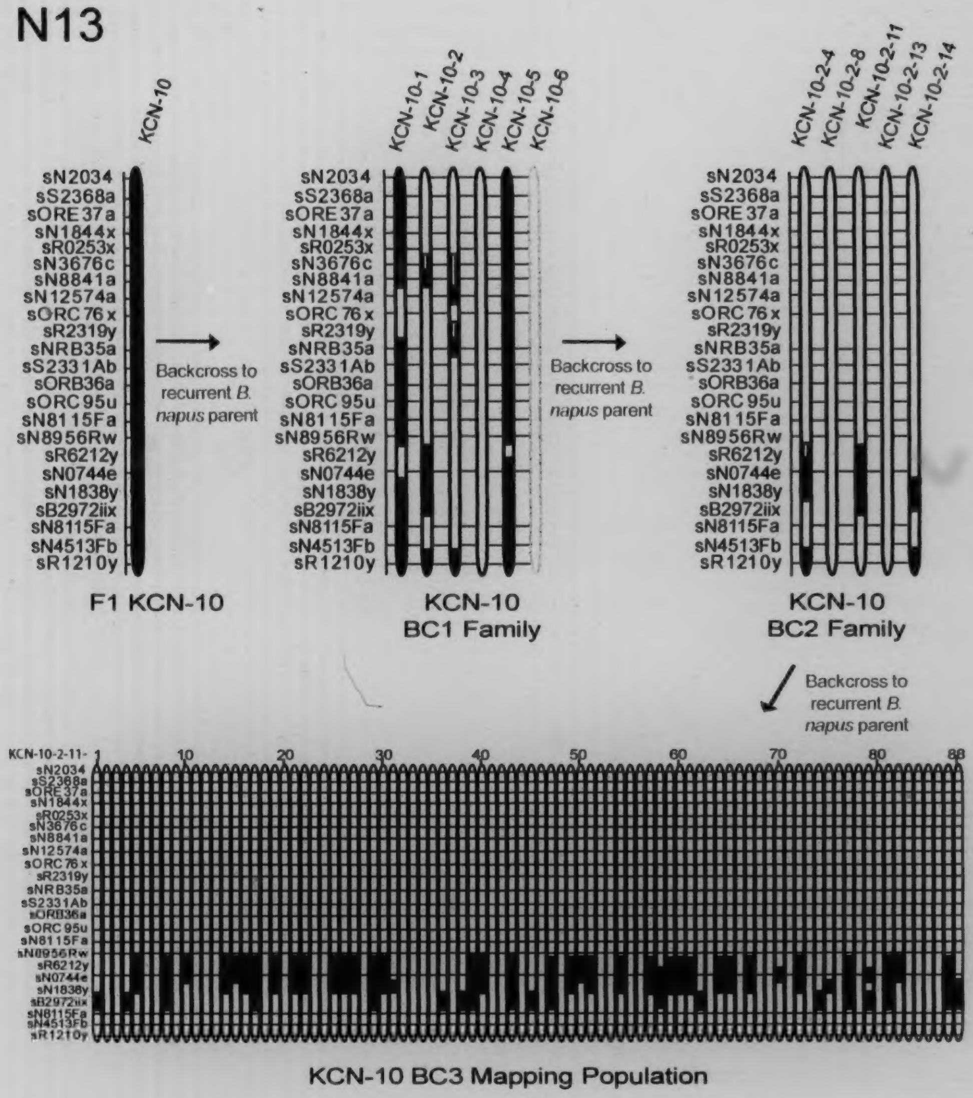

N13
KCN-10
sN2034
sS2368a
sORE37a
sN1844x
sR0253x
sN3676c
sN8841a
sN12574a
sORC76x
sR2319y
sNRB35a
sS2331Ab
sORB36a
sORC95u
sN8115Fa
sN8956Rw
sR6212y
sN0744e
sN1838y
sB2972iix
sN8115Fa
sN4513Fb
sR1210y
Backcross to recurrent *B. napus* parent
F1 KCN-10
KCN-10-1
KCN-10-2
KCN-10-3
KCN-10-4
KCN-10-5
KCN-10-6
KCN-10
BC1 Family
KCN-10-2-4
KCN-10-2-8
KCN-10-2-11
KCN-10-2-13
KCN-10-2-14
KCN-10
BC2 Family
KCN-10-2-11-
1
10
20
30
40
50
60
70
80
88
KCN-10 BC3 Mapping Population

Figure 5-8: Graphical genotypes of N13 for the KCN-10 Sibling families and KCN-10 Mapping Population. Lightly shaded tags indicate the donor parent for the next generation. Labels to the left of the ideograms are SSR markers that were assigned to the linkage group through allele identities on the KCN-5 mapping population and the 61-9 reference population. Markers in the grey boxes are publicly available (Lydiate, D. and Sharpe, A., available through material transfer agreement). Shaded portions of the linkage group indicate the presence of *B.carinata*alleles, unshaded areas indicate the absence of *B.carinata* alleles at the specified loci. The hatched intervals indicate junctions where chromosome disruption ocurred leading to a loss of donor loci beyond the hatched region. Data was unavailable for KCN-10-2-6.

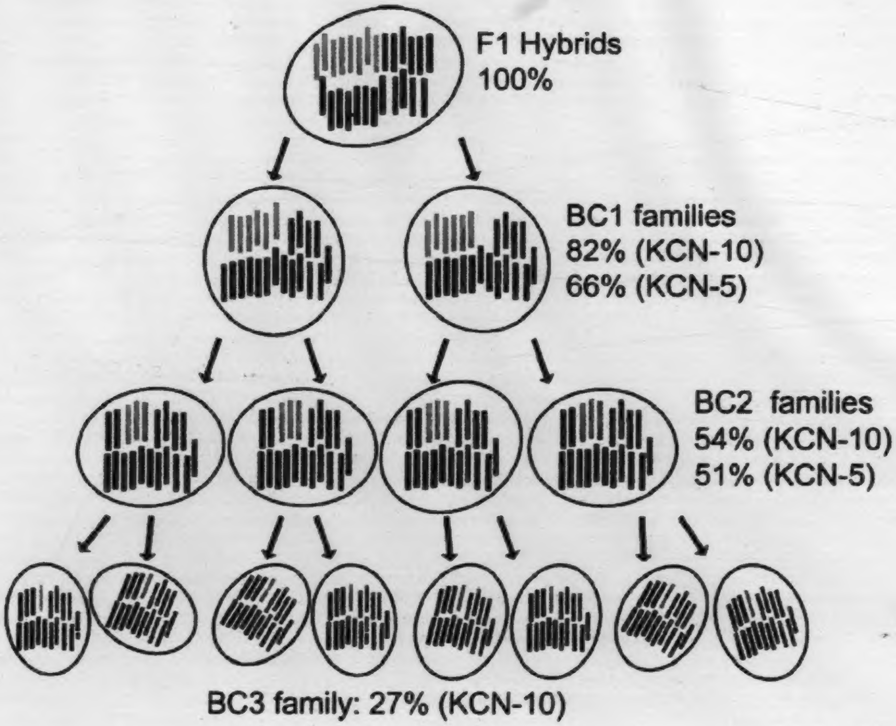

F1 Hybrids
100%
BC1 families
82% (KCN-10)
66% (KCN-5)
BC2 families
54% (KCN-10)
51% (KCN-5)
BC3 family: 27% (KCN-10)

Figure 5-9: Segregation of B genome chromosomes. The average number of B genome chromosomes inherited at each stage of the introgression process is indicated in percentages. Orange ideograms represent the average B genome chromosome compliment at each generation, red chromosomes are A genome chromosomes (assumed to all be present for the purposes of the figure), and black/gray chromosomes symbolically represent the average *B.carinata/B. napus* C genome complements, respectively. Normal Mendelian segregation would have been ~50% for each generation.

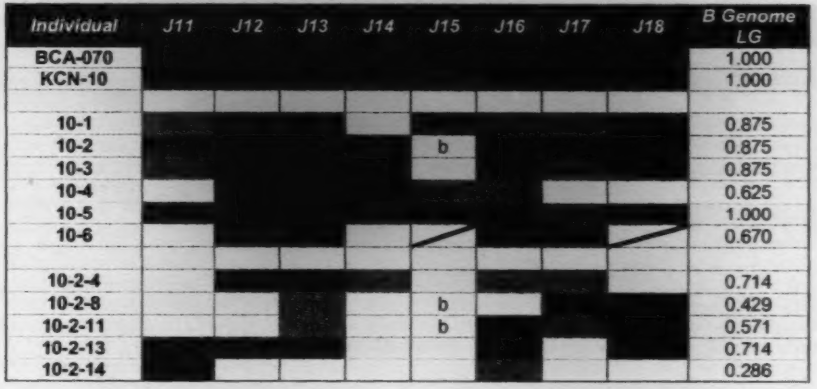
Table 5-2: Allele segregation χ^2 values for the B genome in the BC3 mapping population. LG indicates the chromosome to which core markers were assigned in the KCN-10 mapping population. Chi-squared values (x^2) were calculated using a 1:1 expected allele ratio. The *p*-value was calculated for 1 *d.f.* The S column: "ns" indicates "not-significant", while "*" to "****" indicates significant ($p < 0.05$), to highly significant ($p < 0.0001$) deviations from the H_o. Arrows indicate distortion in favor (↑) of *B. carinata* content, while (↓) indicated higher frequencies of *B. napus* alleles.

Segregation χ^2 values for the B genome of KCN-10 BC3 alleles									
LG	*Marker*	$\chi^2_{1:1}$	*p*	*S*	*LG*	*Marker*	$\chi^2_{1:1}$	*p*	*S*
J13	sJ1322x	23.84	0.00000	*****↓	J17	sB1871x	33.05	0.00000	*****↓
J13	sB3910A	9.67	0.00188	**↓	J17	sJ39119ia	26.45	0.00000	*****↓
J13	sJ1071B	0.02	0.87879	ns	J17	sB1538BB	34.77	0.00000	*****↓
J13	sB2668	7.25	0.00710	**↓	J17	sJ13133a	30.60	0.00000	*****↓
J13	sB0862a	9.89	0.00166	**↓	J17	sJ0143t	27.96	0.00000	*****↓
J13	sB02124B	14.63	0.00013	***↓	J17	sJ1536a	36.47	0.00000	*****↓
J13	sB1752x	11.31	0.00077	***↓	J17	sN8348y	35.64	0.00000	*****↓
J13	sJ1473x	8.45	0.00365	**↓	J17	sB0570a	38.22	0.00000	*****↓
J13	sN12480x	11.86	0.00057	***↓	J17	sJ1086B	30.60	0.00000	*****↓
J13	sB2771a	8.38	0.00380	**↓	J17	sJ4633a	39.12	0.00000	*****↓
J13	sN8348x	10.23	0.00138	**↓	J17	sB0860Ax	33.14	0.00000	*****↓
J13	sJ0266c	12.49	0.00041	***↓	J17	sN1958x	41.26	0.00000	*****↓
					J17	sN9875z	33.84	0.00000	*****↓
J15	sB2334x	0.11	0.74488	ns					
J15	sB31111Bx	0.45	0.50233	ns	J18	sB4727Fa	51.86	0.00000	*****↓
J15	sR6212x	0.05	0.81615	ns	J18	sB0860Aa	30.73	0.00000	*****↓
					J18	sB1728a	34.71	0.00000	*****↓
J16	sJ1071x	9.00	0.00270	**↓	J18	ss2082x	27.43	0.00000	*****↓
J16	sB0202ia	7.53	0.00607	**↓	J18	sJ2013B	27.84	0.00000	*****↓
J16	sB1839x	7.53	0.00607	**↓	J18	sN12624z	30.73	0.00000	*****↓
J16	sB1755B	8.58	0.00341	**↓	J18	sJ3327Rx	31.44	0.00000	*****↓
J16	sB1956a	8.24	0.00409	**↓	J18	sJ0143c	31.25	0.00000	*****↓
J16	sB4727FB	88.00	0.00000	*****↓	J18	sJ0397Ra	30.73	0.00000	*****↓
J16	sB1772ia	11.20	0.00082	***↓	J18	sB3103x	30.60	0.00000	*****↓
J16	sB0273B	0.49	0.48550	ns	J18	sB1956x	38.24	0.00000	*****↓
J16	sJ1505a	3.56	0.05935	ns	J18	sN12624z	30.73	0.00000	*****↓
J16	sJ0502a	5.73	0.01670	*↓	J18	sB0273z	26.30	0.00000	*****↓
J16	sN11516x	11.31	0.00077	***↓	J18	sB2596a	30.73	0.00000	*****↓
J16	sB2545a	27.94	0.00000	*****↓	J18	sN10718x	28.10	0.00000	*****↓
J16	sJ46102y	41.02	0.00000	*****↓					

Table 5-3. Allele segregation x^2 values for loci from three C genome segments in the BC3 mapping population. LG indicates the linkage group to which core markers were assigned in the KCN-10 mapping population. Chi-squared values (x^2) were calculated using a 1:1 expected allele ratio. The *p*-value was calculated for 1 *d.f.* The S column: "ns" indicates "not-significant", while "*" to "****" indicates significant ($p < 0.05$), to highly significant ($p < 0.0001$) deviations from the H_o. Arrows indicate distortion in favor (↑) of *B. carinata* content, while (↓) indicated higher frequencies of *B. napus* alleles.

Segregation χ^2 Values C Genome				
LG	*Marker*	$\chi^2_{1:1}$	*p*	*S*
N11	sN9431x	2.23	0.13559	ns
N11	sN11910x	1.39	0.23827	ns
N13	sN0744e	0.78	0.37699	ns
N13	sN1838y	3.68	0.05501	ns
N13	sR6212y	1.80	0.17971	ns
N17	sORC76c	0.42	0.51763	ns
N17	sN12508iia	0.10	0.74773	ns
N17	sS1949y	1.64	0.20083	ns

Table 5-4: B genome content of the KCN-10 Sibling reference families. KCN-10 Sibling families are aligned on the vertical axis, while B genome chromosomes are aligned along the top of the table. Darkly shaded boxes indicate the presence of whole chromosomes, clear boxes indicate the absence of the chromosomes, and the mid-tone boxes represent chromosomes that were present, but missing one or more terminal segment(s). The segments that were present are denoted by: t=top of the chromosome, m=middle and b=bottom, where 't/m' would indicate that the top and middle of the chromosome were present. In eight cases, only one terminal allele was missing from the chromosome, these segments were denoted by a darkly shaded box, but the inherited portions are still indicated. For J15, 'b' (bottom) in the clear box indicates the presence of the small introgressed span, but the bulk of the chromosome was absent. The 'B Genome LG' indicates the proportion of B chromosomes present over the chromosomes that could have been inherited. The hatched boxes point out two cases where no data was available for the chromosome of interest.



Individual	*J11*	*J12*	*J13*	*J14*	*J15*	*J16*	*J17*	*J18*	*B Genome LG*
BCA-070	■	■	■	■	■	■	■	■	1.000
KCN-10	■	■	■	■	■	■	■	■	1.000
10-1	■	■	■	□	■	■	■	■	0.875
10-2	■	■	■	■	b	■	■	■	0.875
10-3	■	■	■	■	□	■	■	■	0.875
10-4	□	■	■	■	■	■	□	□	0.625
10-5	■	■	■	■	■	■	■	■	1.000
10-6	□	■	■	□	▨	■	■	▨	0.670
10-2-4	□	■	■	■	□	■	■	□	0.714
10-2-8	□	□	▒	□	b	□	■	■	0.429
10-2-11	□	□	▒	□	b	■	■	■	0.571
10-2-13	■	■	■	□	□	■	□	■	0.714
10-2-14	■	□	□	□	□	■	□	□	0.286

5.6. The localization of the *B. carinata* J15 segment

One *B. carinata* J15 span remained in the KCN-10 mapping population. This span was identified with three alleles; sB2334x, sB31111Bx and sR6212x (Figure 5-3). The first two markers (sB2334, sB31111B), were both markers that amplified a single J15 locus in the T-1 AAFC reference map. Furthermore, sB2334 amplified only one allele (*x*) in both BCA-070 and BCA-065 (null plus allele). Allele sB2334*x* was also mapped to J15 in the KCN-5 mapping population (Figure 4-12). In the same vein, sB31111B amplified only one allele in the B genome of the parents; '*a*'-J15 in the KCN-5 mapping population and '*x*' in the KCN-10 mapping population. This marker also amplified two other unassigned alleles from the C genome (Appendix A). The third allele, sR6212x, was informative but not mapped in the KCN-5 mapping population. Accordingly, there was a high probability that this segment was a portion of J15 based on allele size identification and corroborating KCN-5 mapping data.

The J15 segment possessed different characteristics than other maintained B genome chromosome/segments. First, it was smaller than other B genome introgressions observed. Moreover, the J15 alleles sB2334 and sB31111Ba were absolutely linked (0 cM) in the AAFC reference map, and mapped to the tip of J15. The patterns of inheritance observed in this study suggested that terminal B genome alleles were likely to be lost through backcrossing as opposed to being maintained without the central body of the chromosome. Additionally, assay data from the KCN-5 Sibling reference families indicated that KCN-10-1, -4 and -5 maintained these three alleles along with the J15 LG. For the BC1 plant KCN-10-2, the three alleles of interest had contradicting scoring patterns from the remainder of the J15 loci (Figure 5-3).

Finally, the segregation distortion observed for this portion of J15 was equivalent to the ratios observed in the C genome, and were dissimilar to those observed in all instances of B genome inheritance (Table 5-2). The J15 segment was inherited as a whole or in part, in a Mendelian 1:1 ratio (47:41), as were the segments from N11 (45:43) and N13 (48:40) (Figures 5-9 and 5-10). This was also consistent with the other *B. carinata* C genome segments. Additionally, even though at least two of the three alleles were likely to have been tightly linked, traditional recombination between the three alleles was evident (Figure 5-3).

Hence it is probable that a small terminal segment of *B. carinata* J15 was introgressed by recombination or translocation into one of the chromosomes from the A or C genome of *B. napus*. This introgression occurred in the meiosis of the F1 hybrid KCN-10.

5.7. Summary and novel findings

The SSR markers used to characterize the KCN-10 mapping population and its related Sibling reference families were deliberately chosen from pools of characterized alleles. Markers were associated to chromosomes through the use of the AAFC reference maps, the Sibling reference families and the KCN-5 mapping population. These resources made it possible to harvest relevant data from the BC3 mapping population which contained B genome chromosomes that had mostly coincident marker loci, and C genome segments that were difficult to identify due to the maintenance of small introgressions and inflated RF values.

Recombination between B genome chromosomes and chromosomes from the A or C genomes was not observed. B genome loci were generally inherited as coincident blocks. Recombination values for B genome chromosomes involved in conventional crosses (AAFC reference populations) showed RF values at least ten-fold higher than those observed in the KCN-10 mapping population. Even so, the observed *p*RF values of the KCN-10 population represented the loss of terminal alleles and were not the result of traditional recombination. Conversely, the C genome segments underwent a two-fold increase in recombination when compared to *B. napus* intraspecific crosses.

The construction of graphical genotypes was crucial to the illustration of B genome chromosome inheritance. These diagrams showed the maintenance of whole B genome chromosomes and the progressive loss of terminal segments through three generations of backcrossing. The diagrams were also used to detect the non-reciprocal translocation of a small terminal segment of J15, which was transferred to the A or C genome of *B. napus* during a meiotic event in KCN-10. In contrast, the *B. carinata* C genome was inherited in small dispersing fragments as would be expected via homologous recombination.

Segregation of the B genome chromosomes and loci were also observed. While the BC1 and BC2 families showed some evidence of preferential retention of B genome chromosomes, the BC3 B genome chromosomes were inherited with an average frequency of 27%. This observation was reflected in the allelic segregation distortion observed for the KCN-10 mapping population. The loci associated with the B genome in the mapping population were inherited at frequencies that were significantly different than the Mendelian 1:1 segregation ratio that was expected. B genome alleles, even the central conserved regions of the chromosomes, were preferentially lost during BC2 meioses. Conversely, the C genome loci were inherited in a 1:1 ratio at each generation.

Additionally, the KCN-10 population and its progenitor families, are a large resource of interspecific germplasm with known B/C genome content. This germplasm segregates for several characteristics, including blackleg resistance, and will be used in further characterizations.

6. General conclusions and novel findings

This project described the genetic events that corresponded to a backcrossing introgression approach which is frequently used in the breeding of many crop species, including the *Brassica*. *Brassica napus* and *Brassica carinata* were crossed to develop several lineages of interspecific germplasm. This germplasm was then used to characterize the genetic changes that accompanied backcrossing and was used to monitor the tempo of *B. napus* reconstitution following interspecific backcrossing. A number of genetic characteristics were important to the type of crossing undertaken in this project. Primarily, both *B. napus* and *B. carinata* are polyploids that share one subgenome (C), but differ between subgenomes A and B.

The conclusions related to the behavior of the SSR primer pairs, and the behavior of the *Brassica* subgenomes during the *B. napus* X *B. carinata* crosses, are described in sections 6.1 to 6.8. In addition to these observations, several other aspects of this thesis are significant and novel. To date, this research describes:

- The first *B. carinata* X *B. napus* interspecific map.
- The first use of both genetic and junction maps to characterize the genomes of successive generations of a cross.
- The largest number (150) of junction maps constructed to describe a series of inter- or intraspecific crosses.
- The first identification of the *B. carinata* genome with C and B genome molecular markers.
- The first molecular evidence that *Brassica* B genome chromosomes fail to recombine with the A/C genomes during introgression.
- The first evidence that the *B. napus* and *B. carinata* C genomes can recombine via homologous recombination, despite being evolutionary diverged.

6.1. Stringent selection of molecular markers for the identification of the *Brassica* subgenomes increased the accuracy and efficiency of mapping

Selecting *Brassica* markers based on polymorphic alleles has been an essential part of mapping work. More recently, markers have been chosen with added diligence amid hopes of yielding more informative data. In the case of mapping work leading to map-based cloning, marker selection based on putative gene homologues and conserved structural or amino-acid motifs has been employed (Silitto *et al.*, 2000; Mayerhofer *et al.*, 2005). Also, molecular maps are currently available for the vast majority of crop species and can be used to align new maps, anchor markers, and determine collinearity. These resources, conscientiously applied, shortened the time needed to develop a new linkage map and increased the efficacy of the map. The more stringent characterization of markers prior to their application, as carried out in this project, was useful for efficient labeling of genomes/ chromosomes, interpreting amplification patterns, anchoring new alleles, and constructing linkage groups despite highly distorted recombination.

It was evident that markers designed to be genome specific in certain germplasm could not be assumed to be genome specific even when presented with related germplasm. The A and C *Brassica* genomes share many collinear regions and are considered to be homeologous to one another (Parkin *et al.*, 2003). Likewise, homology between the A and B genomes has been established (Plieske and Struss 2001), and to a lesser extent A, B and C genomes (Quiros *et al.*, 1991; Truco *et al.*, 1996). This supports the concept that the *Brassica* genome has undergone extensive duplications followed by chromosomal rearrangements (Sun *et al.*, 2006; Parkin *et al.*, 2003; Truco *et al.*, 1996; McGrath *et al.*, 1990; Hu and Quiros 1991).

Of the markers used in this study, it was estimated that 19.5% of the markers considered to be A/C genome resources also amplified loci in the B genome of BCA-065/BCA-070 *B. carinata* germplasm. More importantly, 4.5% of the markers that were considered to be B genome specific, amplified loci in the BCA-065/BCA-070 *B. carinata* C genome. This is of considerable importance, as an absence of data on these alleles could lead to erroneous reporting of B genome segments of a non-recurrent parent integrating into the C genome, which may be more common than appreciated.

Resolving the identity of amplified alleles was achieved through the use of multiple molecular maps with aligned characterized markers, and the production of screening panels for each marker that included genomic samples of the *Brassica* subgenomes. This information made allele assignment to specific chromosomes more rapid, aided in the identification of allelic and non-allelic loci, and increased my ability to discriminate between *B. napus/B. carinata* C genome recombination versus putative B genome integrations into the C genome. Also, it was important to be sure that terminal loci on each linkage group were being amplified. This made the detection of the KCN-10 J15 translocation possible. Without such knowledge, meiotic events on the chromosome tips relative to the interior may have been undetected.

Many of the alleles could be attributed to specific chromosomes prior to their use on mapping panels. This pre-selection permitted better planning of chromosome coverage, required fewer molecular markers to achieve equivalent marker saturation, and drastically reduced the number of unassigned loci.

6.2. Allowing early generation hybrids to set seed resulted in parthogenic offspring and did not increase the probability of introgressions

Often, breeding strategies where introgressing traits are concerned focus on bringing initial germplasm through by seed. It has been reported that allowing plants to set seed can select for increased fertility (more harmonious chromosome pairing) and faster recurrent parent reconstitution (Stringam *pers. comm.* 1999; Struss *et al.*, 1991; Eenink 1974; Dixelius and Forsberg 1999). During this research, F1 plants that were allowed to set seed from backcross bud pollinations generated seed at a similar rate to the generation of viable ovules rescued for tissue culture, but were likely to generate parthogenic seed (data not shown). Hence, this approach would not be the most effective method for generating hybrid germplasm in these species.

Postzygotic speciation theories, (Rieseberg *et al.*, 2000), would predict that stable introgressions would be more prevalent if F2 seed were able to mature on early generation hybrids. However, hybrid germplasm was sterile and the ability of second and third generation hybrids to produce fertile self seed and backcross seed was negligible. Additionally, in the KCN-5 mapping population, there was no relationship between B genome chromosome content and self-fertility (F2 seed), nor was there any particular chromosome that associated consistently with fertility/sterility. There was also no relationship between the number of B chromosomes and BC seed generation. This suggests that early hybrids of all fertility should be investigated for introgressions and that fertility was not a reliable estimation of incorporated B genome content.

In addition, the *B. carinata* parent being the initial female donor (KCN-5 lineage) versus the male donor (KCN-10 lineage), had no discernable effect on the pattern or frequency of B genome chromosome inheritance.

6.3. Recombination in the B genome was consistently repressed

It has been reported that the average ratio of genetic to physical distance varies widely across chromosomal regions within species (Deepak and Kulvinder 2005), between male and female meioses (Nelson *et al.* 2005), as well as across taxonomic groups. The results of this research indicated that for hybrids, RF values are heavily influenced by the presence or absence of homeologous and homologous pairs in hybrid nuclei. It would seem that although regions of collinearity have been established between *Brassica* A, B and C chromosomes (Parkin *et al.,* 2003; Plieske and Struss 2001; Quiros *et al.,* 1991; Truco *et al.,* 1996), metaphase bivalents/ multivalents have been observed when new *Brassica* polyploids are established (Struss *et al.*, 1991; Maoteng *et al.*, 2004; Chandra *et al.*, 2004; Dixelius, 1999), and hybrid phenotypes observed, (Choudhary *et al.*, 2002), one cannot assume that genetic recombination is occurring. For example, Maoteng *et al.*, (2004) and Li *et al.,* (2005) stated that the occurrence of trivalents and quadrivalents at diakinesis in *B. napus/B. carinat*a interspecific hybrids suggested that A/B and A, or B/C^n and C^c homologous pairing and exchange was occurring. However, the molecular analysis presented here indicates that although the B genome is collinear with some A and C genome regions, recombination between the B genome and the A/C is unlikely.

When KCN-5 and KCN-10 mapping population B genome *p*RF values were compared to the same loci found in the established interspecific (T-1, AAFC, Axellson *et al.*, 2000) map, recombination was depressed by twelve-fold. However, as shown in the junction maps for each B genome chromosome, the *p*RF values represented terminal loss of B genome loci and not recombination. Furthermore, if the linkage maps were further saturated, the disparity between the inter-, and intraspecific maps would become greater. Table 6-1 shows the comparative RF/*p*RF values for each characterized chromosome. These values were discussed in Chapter 4 and 5 and represent only the shared loci between the test populations and the AAFC reference maps.

Table 6-1: Recombination frequencies between conserved loci in the KCN-5, KCN-10 and AAFC reference maps. RF/*p*RF values are shown in cM and use only shared loci to determine the comparative RF values. Where two RF/*p*RF values are listed, the first corresponds to the KCN-5 mapping population relevant RF/*p*RF, and the second corresponds to the loci shared by the KCN-10 mapping population. A double asterisk highlights the J15 segment (integrated into the A or C genome) and is not included in the total B genome *p*RF calculations. The triple asterisk indicates that multiple unlinked segments were present, so three linked segments (encompassing the same loci) were used for comparison. In the case of N16, the LG was not present in the 61-9 population and there were too few linked alleles in common between the 72-8 map to make a comparison.

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
J11	11.5	n/a	62.9	**N11**	204.6	13.7*	114.8/ 8.9
J12	2.7	n/a	105.5	**N12**	124.3	27.5*	64.1/ n/a
J13	3.1	2.3	71.2/ 44.2	**N13**	284.5	49.0*	96.8/ n/a
J14	12.7	n/a	69.9	**N14**	199.2	n/a	81.2
J15	13.3	35.3**	95.0/ 0	**N15**	49.0***	n/a	20.2***
J16	8.7	5.8	55.6/ 60.6	**N16**	n/a	n/a	n/a
J17	9.0	3.7	49.3	**N17**	116.8	33.8*	57.9/ 10.2
J18	13.2	13.9	73.8/ 66.6	**N18**	93.2	34.3*	53.4/ n/a
Total	*74.2*	*25.7*	*583.2/ 220.7*	**N19**	n/a	20.7*	n/a
				Total	*1071.6*	*182.0*	*488.4/ n/a*

6.4. The C genome of *B. carinata* was introgressed via homologous recombination and the resulting RF values were consistently inflated

Contrary to the results derived for the *B. carinata* B genome, the *B. carinata* C genome chromosomes were introgressed into the *B. napus* C genome as would have been predicted by Mendelian segregation. The graphical genotypes of this process show the introgressed segments in the F1, BC1-BC3 plants (Figures 4-14, 5-7, and 5-8) and illustrate the gradual breaking up of these segments as backcrossing proceeded.

In both mapping populations, no consistent segregation distortion was observed for the loci on the C genome. Thus if a locus was present in the progenitor plant, it was reliably inherited 50% of the time in the progeny.

Due to the more advanced backcross of the KCN-10 mapping population, it was challenging to confidently resolve all of the C genome introgressions. This was due to the low number of *B. carinata* C genome segments remaining, and the inflated RFs between the *B. carinata* and *B. napus* C genome. There was no evidence of A/C homeologous recombination in the cross, although the A genome was genetically invisible due the use of a recurrent *B. napus* parent. Homeologous recombination does occur in *B. napus,* accounting for just less than 1% of recombination events in resynthesized *B. napus* (Udall *et al.*, 2005). Hence, there is a possibility that undetected A/C recombination events occurred in the KCN-5/ KCN-10 germplasm.

When RF comparisons were made between both mapping populations and the AAFC reference maps, the C genome RF values were consistently approximately 2.2 fold higher than the AAFC maps generated via intraspecific crosses (Table 6-1). Due to the inflated RFs, many C genome segments in the reduced shared subset used to generate the comparative figures were unlinked. If the *B. carinata* C genome could be further saturated, the difference between the AAFC reference RF values and the experimental RFs would be larger. Therefore the interspecific C genome RF values could be greater than the 2.2 fold determined here.

The finding of enhanced C genome recombination in this cross was unexpected considering the C genome of *B. carinata* might have diverged significantly from that of *B. napus*. The molecular interactions that led to this phenomenon have yet to be determined. Natural interspecific hybridizations would however experience strong selection in favor of increased chromosomal rearrangements to increase the genetic variation of a novel, but small population.

6.5. B genome chromosomes were preferentially maintained in BC1 hybrid germplasm but were preferentially lost during BC2 meioses

It was anticipated that each B genome chromosome ought to be present in each F1 progenitor plant, whereas the expected frequency of inheriting a specific B genome LG in the BC1 plants ought to be 50%. However, for the BC1 germplasm it was determined that the B genome chromosomes were preferentially retained in BC1 generation hybrids.

In the case of the KCN-5 and KCN-10 lineages, all B genome LGs were present in the F1 hybrids as expected. For the average KCN-5 BC1 plant, 5.28 LGs (66%) of the eight chromosome B genomes (median 75%) were inherited , while the same value for the KCN-10 BC1 plants was 6.56 (82%, median 87.5%). For BC2 plants, the average KCN-5 BC2 plant had a 51% B genome retention frequency, and the average KCN-10 BC2 plant had a similar retention of 47.5%. However, in the case of KCN-10-2, this progenitor BC1 plant did not contain all of the B genome LGs. After taking this into consideration, the average retention rose to 54.3%. Thus, BC2 plants exhibited approximately the expected Mendelian 50% inheritance frequency (Figure 5-9).

The same values for the plants comprising the BC3 KCN-10 mapping population were 1.4 (35%) B genome LGs per BC3 plant, with no plant inheriting all four B genome LGs present in KCN-10-2-11.

In terms of a bias for, or against, the inheritance of any particular B genome LG, the frequency of inherited B genome LGs in the KCN-5 BC1 plants ranged from 50% (J17) to 77.5% (J12). Only LG J12 was inherited significantly ($p^{x2} < 0.05$) more often in the BC1 plants, while J17 was incorporated more often in the small

KCN-5 BC2 family. For the KCN-10 lineage, The BC1 plants inherited J12 and J13 more frequently that the other B genome LGs (6/6 times for each), and the BC2 B genome chromosomes were not inherited in a significantly different ratio than would be expected.

The BC3 KCN-10 mapping population exhibited a lower conservation ratio than the 1:1 expected Mendelian segregation ratio for each B genome chromosome that was present in the progenitor BC2 plant. The remaining B genome chromosomes (J13, J16, J17 and J18) were inherited with an average frequency of 27%, and ranged from 20% (J17 and J18, Figures 5-7 and 5-8) to 33% (J13, Figure 5-3). These inherited B genome LG values were below the 1:1 ratio that was observed in the BC2 generation. Analysis of allele segregation distortion confirmed the observations that the B genome alleles on J12, J15 and J16 were inherited more frequently in the BC1 plants, but the conservation of alleles on the retained B genome chromosomes J13, J16, J17, and J18 were significantly lower than the expected 1:1 ratio in the BC3 mapping population.

Both BC2 populations used in this analysis were too small for robust statistical analysis. Also, the KCN-10 BC2 family was selected based on resistance to *L. maculans*. Hence, some bias concerning B genome LG maintenance could have occurred in the KCN-10 lineage. Nevertheless, B genome LGs were clearly inherited at higher than the expected proportions in both unselected and selected early generation hybrids (BC1), but the trend reversed when BC3 plants were characterized.

Additionally, it was not determined if the B genome chromosomes were segregating as aneuploid chromosomes, or whether other genome linkage groups were excluded or could act as partners in normal segregation. It was determined that in F1, BC1 and BC2 individuals there was a high probability of all B genome LGs being present. Since SSR markers do not efficiently differentiate between single or double copy alleles, it was not possible to prove that aneuploid chromosomes were present. However, as all of the BC1 plants contained the alleles from all of the C genome LGs as well as the B genome chromosomes, it is most likely that the B genome chromosomes were present as aneuploid chromosomes (Chevre *et al.*, 1991;

Roussel *et al.*, 1999), but normal *B. napus* chromosome compliments have been observed with interspecific morphologies (Schelfhout *et al.*, 2006).

In opposition to the B genome, C genome segments and alleles were inherited in a 1:1 ratio in BC1, BC2 and BC3 generations. In fact, the KCN-5 and KCN-10 mapping population segregation ratios of introgressed *B. carinata* C genome loci was so distinct from the inheritance of B genome loci, that scoring matrix data could be ascribed to the C or B genome based on segregation ratios. For example, consideration of segregation distortion supported the assignment of a small translocation from J15 to a C or A genome LG in the KCN-10 mapping population.

6.6. A terminal J15 segment was introgressed into the A or C genome of *B. napus*

A small terminal region of J15 was identified by allele assignments on the AAFC reference maps and the KCN-5 map. The probability that the segment was an incorrectly assigned *B. carinata* C genome region was 0.0007. The three J15 loci were originally located at the tip of J15 and were segregating in a 1:1 ratio in the KCN-10 mapping population. Additionally, it was consistently observed that terminal sections of B genome chromosomes were being lost during backcrossing. Further consideration of the allelic segregation distortion, and the presence of recombination between the three loci following the putative introgression, suggested that this small terminal region of J15 had introgressed into the A or C genome during an F1 meiosis. Although it is more probable that this introgression occurred in the A genome of *B. napus*, the inflated RF values observed in the C genome made establishing linkage between C genome segments difficult. Therefore, a C genome incorporation of the J15 segment could not be ruled out.

The J15 segment introgression could have been the result of a nonreciprocal translocation or the result of a scavenged chromosome breakage. Out of 150 genetically characterized plants (F1 to BC3), this was the only observed transfer of B genome material to the genome of *B. napus*. Since it was probable that terminal loci on all B genome chromosomes were monitored (AAFC reference maps), it was unlikely that another such introgression was missed due to a lack of B genome resolution.

Given these results, when using interspecific/intergeneric crossing in the *Brassica* as a vehicle for transferring traits from one species to another, it would seem that the desired trait would need to be on a shared subgenome, or would need to be a simple trait located on a terminal region (tip) of the unshared genome chromosome. However, if a trait is complex (multiple gene pheontype) or was centered on a chromosome, it is unlikely that it would be introgressed into a non-homologous genome through conventional breeding methods.

6.7. Recombination of B genome chromosomes with the A or C genome of *B. napus* was not observed

The alignment of loci and chromosomes through the use of graphical genotypes was useful for determining the architecture of the B and C genomes. Although scoring data and linkage maps are typically used alone to describe the LGs of mapping crosses, linkage maps alone inadequately described the LGs and recombination events/chromosome breaks in the hybrid crosses. Most recently, Rieseberg *et al.* (2000) described the graphical genotypes (junction maps) of two BC1 individuals derived from a cross between *Helianthus petiolaris* and *Helianthus annuus*. This thesis presents the construction of 150 graphical genotypes.

Through the use of these junction maps, it was consistently observed that B genome chromosomes were maintained as independent non-recombining chromosomes through backcrossing. It was apparent that the measured B genome *p*RF values were a reflection of terminal allele loss, and not of classical recombination. Thus, the observed modifications to the B genome mainly consisted of the loss of chromosome tips in each of the characterized lineages for all B genome LGs. This phenomenon was also observed by Hu and Quiros (1991) in *B. rapa*/*B. oleracea* addition lines. Occasionally, a small internal segment was inherited, but these segments were conserved in the mapping population, possibly indicating the location of the centromere, or a relic centromeric sequence. On occasion, a terminal B chromosomal segment was conserved (Figure 4-10; KCN-5-

2-24 and -33). These instances could represent the recognition of relic centromeric sequences, or could be terminal non-reciprocal translocations. Molecular analysis of the next generation for these individuals would be required to determine if these terminal loci had been translocated.

6.8. Drawing conclusions in regard to introgressed B genome germplasm

Many attempts have been made over the last three decades to exploit *Brassica* B genome sources of blackleg resistance for use in *B. napus*. Transfer of *B. juncea* blackleg resistance to *B. napus* through the creation of interspecific hybrids was first described by Roy in 1978. Early cytological studies of these hybrid-derived resistant lines indicated that they possessed the correct number of chromosomes for *B. napus* ($2n = 38$), although true breeding resistance could not be obtained (Roy, 1984). However, Rimmer and Van den Berg's (1992) later examinations demonstrated that the majority of lines were actually aneuploid. Material from these crosses, and other interspecific hybrids, has been used in on-going attempts to characterize the inheritance of B genome blackleg resistance. As discussed earlier, many groups have described B genome introgressions into the genome of *B. napus*, but to our knowledge, none of them have been developed into registered cultivars (Plieske *et al.*, 1998; Dixelius 1999; Roussel *et al,*. 1999; Pang and Halloran 1996; Roy *et al.*, 1997; Chevre, 1999; Batra *et al.*, 1990; Brown *et al.*, 1997; Earle *et al.*, 1992; Gundimeda *et al.*, 1992; Landgren and Glimelius 1994; Liu *et al.*, 1995; Nanda Kumar and Shivanna 1993; Sundberg 1998; and Glimelius 1991).

Although a B genome incorporation was observed (the J15 segment) during this research, the results presented in this thesis could be used to explain the difficulties of developing introgressed B genome material and reveal;

a) Why introgressed B genome loci do not seem to persist through to cultivar generation.

b) How it would be possible to erroneously conclude that B genome chromosome segments are being incorporated into the A/C genome of *B. napus*.

6.8.1 Introgressed B genome loci could actually be entire B genome chromosomes that are then lost in breeding

Due to the lack of recombination between the B genome chromosomes and A/C chromosomes, B genome loci are essentially coincident through the interpretation of mapping software functions. Hence, the B genome chromosomes could be perceived as physically small introgressed segments. One could conclude that a number of molecular markers (along with a trait of interest) from a B genome LG had been inherited and was segregating in a population. This phenomena has been previously been reported as introgression (Barret *et al.*, 1998; Saal and Struss, 2005).

Additionally, as aneuploid or non-recombining chromosomes, the B genome chromosomes are expected to be lost during the breeding process. As shown in the experimental mapping populations described here, early generation hybrids (F1 to BC2) would seem to retain their B genome chromosomes, and therefore blackleg resistance, at high rates. After the early stages of the breeding process, breeding populations would segregate for blackleg resistance. According to the results presented here, populations at about the BC3 generation would retain blackleg resistance about 30% of the time. In fact, this is what was observed by Plieske *et al.*, (1998), who attributed hot greenhouse conditions for the unexpected inheritance patterns they observed. Over time, it would be expected that non-recombining chromosomes would be lost, and most importantly, any linkage drag associated with the retained chromosomes could not be separated from blackleg resistance.

6.8.2 B genome specific markers were not specific to the B genome all of the time

When genome specificity of the SSR markers was explored, 4.5% of the markers that were considered to be B genome specific amplified loci in the BCA-065/BCA-070 *B. carinata* C genome. Therefore, although these markers only amplified B genome loci in standard germplasm, when challenged with novel germplasm, the molecular markers amplified other alleles in the C genome of *B.*

carinata. Since the C genome of *B. carinata* introgressed via homologous recombination into the *B. napus* C genome, it could appear as though B genome introgression was occurring.

6.8.3. Segregation distortion in the B genome links B genome chromosomes into one LG

It is common practice to use mapping software for the generation of linkage maps, QTL analysis, and marker-assisted selection. There is a variety of software available for these functions, but the statistical tools involved in the calculation of RF values are functionally equivalent. During interspecific crossing in the *Brassica,* it was shown that, while mapping software was helpful for the generation of RF values between loci, the information was misleading unless the alleles were assigned to specific chromosomes beforehand. For example, Figure 6-1 shows the MapDisto and MapMaker output map for a small subset of KCN-5 mapping population scoring matrix data (Lander *et al.*, 1987; Lorieux, 2002). The sample contained scoring data from N13, J11, J14 and J18. Most of this data subset was portrayed as a single linkage group (N13, J11, J14 and J18), and was one out of two large LGs generated with the software packages. The B genome LGs are displayed as small regions (depressed recombination), while the physically smaller C genome sections are displayed as statistically larger (inflated recombination). If this were a linkage map developed for marker assisted selection, it would have been less saturated (less clustering of loci in the B genome), and the 178 cM map generated for this LG would have appeared to be normal. Therefore, if a desirable trait was located on J18, for example, one could assume that the 13.2 cM J18 trait was situated on N13.

Mapping software can generate these types of graphics for several reasons. Primarily, it is because the RF values of both the C and B genomes are highly skewed relative to the physical distances involved. Additionally, the segregation distortion of loci on the B genomes is not 1:1. For the BC1 plants, loci were preferentially inherited (more '+' scorings than '–' scorings), this artificially links the B genome LGs together. This phenomenon is exacerbated in later generations. For the BC3 mapping populations, excessive '–' scorings linked all of the B genome

LGs together.

These factors, when combined with a random application of molecular markers, QTL mapping or bulk segregant analyses, could lead to erroneous interpretations of introgression data.

6.9. Future considerations

One of the original objectives of this project was to locate the gene(s) responsible for resistance to *L. maculans* within the B genome. Both the KCN-5 and KCN-10 mapping populations segregated for blackleg resistance and the applied molecular markers were informative, but the results were inconclusive. The B genome segments did not recombine readily with homeologous regions of their host genome (A/C). This resulted in the appearance of close linkage between loci on the introgressed segment despite very large physical distances, limiting the utility of molecular markers for applications such as mapping, positional cloning or breeding. Blackleg resistance was localized to one B genome chromosome- J18, but J18 molecular markers and resistance/susceptible scorings could not be resolved (data not shown). Furthermore, I would speculate that similar introgression projects have experienced the same pitfalls. Thus, the most effective means of transferring B genome derived blackleg resistance to *B. napus* would be to first fine-map the gene(s) in an intraspecific B genome *Brassica* cross, and then bring the trait across to the A or C genome by means of a transgenic approach. Christianson *et al.* (2006) used a susceptible *B. juncea* X resistant *B. juncea* cross to localize blackleg resistance within J13 (dominant) and J18 (recessive) of the B genome. Hence, map based cloning of these genes would be a practical next step. While the germplasm generated in this study is unsuitable for map based cloning, the analysis of the dominance/recessive nature of the two genes involved (J13 and J18) is ongoing.

In order to increase blackleg resistance in canola quality oil producing species, the continued agronomic improvement of *B. juncea* (AABB) is a viable alternative, as would be moderating the factors responsible for the repression of homeologous recombination in *B. napus* germplasm.

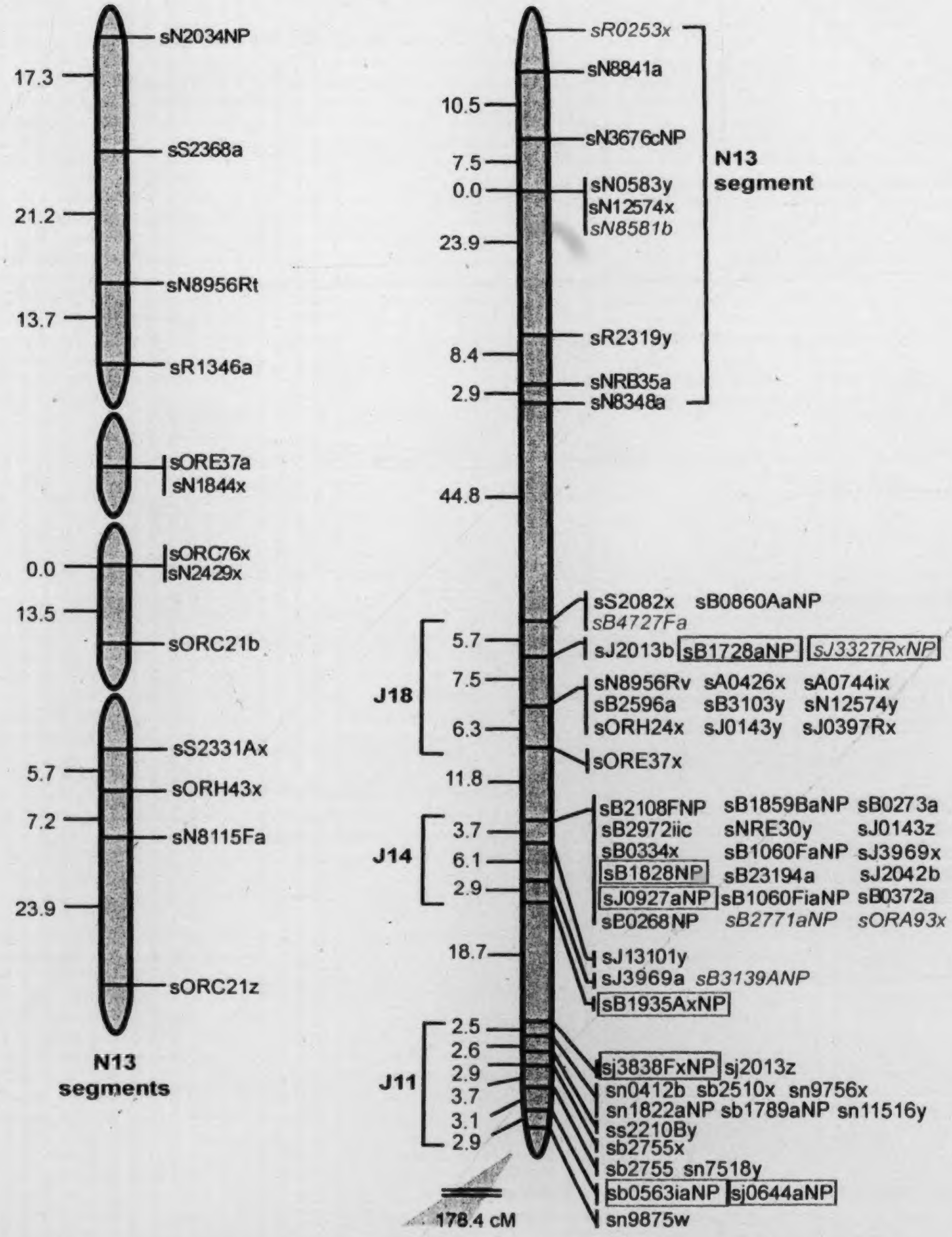
Although two hybrid lineages were characterized with molecular markers during the course of this project, hybrid germplasm from eight other lineages is available for characterization. Some morphological traits have been initially observed to associate with particular B genome chromosomes, and these families could be used to elucidate which chromosomes confer which traits. Some of these traits would be considered valuable (*alternaria* disease resistance), while others would be classified as negative linkage drag (flower deformities). Furthermore, the relationship between phenotype variations of certain B genome alleles within different genetic backgrounds, and their relationship to heterosis should be sought.

Furthermore, it has not been determined if the KCN-5 or KCN-10 germplasm is aneuploid for the incorporated B genome chromosomes. The small size of chromosomes in the *Brassica* is problematic for any cytological study and there is a lack of suitable cytological preparation techniques to identify introgressed segments and translocations (Snowdon, 2007). Distinction between A and C genomes of *B. napus* is currently not possible due to the high homology of the genomes. However, the B genome can be readily distinguished from the A and C genomes using genomic in situ hybridization (Schelfhout *et al.*,2006; Snowdon *et al.*, 2000; Wang *et al.*, 2004). Genomic in situ hybridization (GISH) or fluorescence in situ hybridization (FISH), in combination with the junction maps, could provide a more complete image of the changing architecture of hybrid genomes as they were advanced.

The C and B genomes of the germplasm used in this study generated very different recombination values. There are various groups researching the structure and control of the proteinaceous synaptonemal complex involved in recombination. Specifically, there may be a gene(s) similar to *Ph1* in wheat that suppresses homeologous chromosome pairing (Riley and Chapman 1958). In fact it is the successful suppression of *Ph1* that allows for alien introgressions in wheat breeding (Snowdon, 2007). In *B. napus*, the locus *PrBn* was found to suppress non-homologous pairing in both diploid and haploid meioses (Jenczewski *et al.*, 2003). When the loci involved in the regulation of meiotic pairing control are elucidated, it may be possible to alter the dynamics of recombination to allow homeologous recombination between the A/C and B genomes of *Brassica*.

It is also not clearly understood why certain chromosomes experience segregation distortion. Although distortion may also be the result of chromosomal segregation distortion, the possibility that certain loci may be more heritable cannot be ignored. Segregation distortion in hybrids has been previously reported. In *Helianthus*, segregation distortion has been observed at 7–13% of loci in intraspecific mapping populations compared to 23–90% of loci in interspecific crosses. Not only are distorted ratios prevalent, they can also be acute. For example, segregation ratios that were skewed 12:1 in favor of 'wild' alleles have been reported in crosses between cultivated pearl millet (*Pennisetum glaucum*) and one of its wild relatives (*P. violaceum*). Thus, hybrid progeny may receive more alleles from one parent than would be expected under traditional rules of segregation (Rieseberg and Linder 1999).

Both *B. napus* and *B. carinata* are ancestral polyploids, genomic allopolyploids and share collinear regions with each genome. As such, *B. napus/B. carinata* hybrids probably exhibit all of the molecular and chromosomal irregularities that befall new polyploids. These likely include changes in methylation/gene expression, induction of transposons, the uncoupling of co-adapted gene complexes by recombination, and incompatibility interactions among many loci (Lukens *et al.*, 2006; Song *et al.*, 1995; Auger *et al.*, 2005; Liu and Wendel, 2000; Bircher *at al.*, 2005; Rieseberg *et al.,* 2000). The molecular grounds for hybrid vigor, heterosis and these changes in gene expression in early generation hybrids have not yet been determined. Although epigenetic factors are recognized by breeders as being significant contributors to cultivar development, there are few strategies of how to manipulate, track or compensate for them during breeding. Hence, the creation of novel polyploids in (A/C X B genomes) *Brassica* should be considered comparable to interspecific/intergeneric crossing, and has less in common with intraspecific breeding.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17.3
sS2368a
21.2
sN8956Rt
13.7
sR1346a
sORE37a
sN1844x
sORC76x
0.0
sN2429x
13.5
sORC21b
sS2331Ax
5.7
sORH43x
7.2
sN8115Fa
23.9
sORC21z
N13
segments
sR0253x
sN8841a
10.5
sN3676cNP
7.5
N13
segment
0.0
sN0583y
sN12574x
sN8581b
23.9
sR2319y
8.4
sNRB35a
2.9
sN8348a
44.8
sS2082x sB0860AaNP
sB4727Fa
5.7
sJ2013b sB1728aNP sJ3327RxNP
J18
7.5
sN8956Rv sA0426x sA0744ix
sB2596a sB3103y sN12574y
6.3
sORH24x sJ0143y sJ0397Rx
sORE37x
11.8
sB2108FNP sB1859BaNP sB0273a
3.7
sB2972iic sNRE30y sJ0143z
J14
6.1
sB0334x sB1060FaNP sJ3969x
sB1828NP sB23194a sJ2042b
2.9
sJ0927aNP sB1060FiaNP sB0372a
sB0268NP sB2771aNP sORA93x
18.7
sJ13101y
sJ3969a sB3139ANP
sB1935AxNP
2.5
2.6
sj3838FxNP sj2013z
J11
2.9
sn0412b sb2510x sn9756x
3.7
sn1822aNP sb1789aNP sn11516y
3.1
ss2210By
2.9
sb2755x
sb2755 sn7518y
sb0563iaNP sj0644aNP
178.4 cM
sn9875w

Figure 6-1: Mapping software output linkage map of partial data from the KCN-5 mapping population. The diagram shows the linkage map generated using MapDisto with scoring data from N13, J11, J14 and J18 (Lorieux, 2002). The chromosome designations were assigned after analysis of the alleles. Labels to the right of the ideograms are SSR marker loci, RF/*p*RF values and LG designations are on the left. The graphic depicts how the distinction between the C genome and the B genome chromosomes would be difficult to determine without prior marker characterization.

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List of Appendices

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